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# NUDIBRANCHS OF THE ROSS SEA, ANTARCTICA: PHYLOGENY, DIVERSITY, AND DIVERGENCE

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# NUDIBRANCHS OF THE ROSS SEA, ANTARCTICA: PHYLOGENY, DIVERSITY, AND DIVERGENCE

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**ABSTRACT**

The Southern Ocean (SO) surrounding Antarctica is extremely cold and geographically isolated. The phylogenetic affinities of only a few SO taxa have been examined in detail; in these, a high degree of endemism and radiation within the SO has been established using molecular phylogenetic methods. In order to address these Antarctic paradigms, we used Bayesian inference to construct phylogenetic trees of nudibranch molluscs based on mitochondrial cytochrome-c oxidase I (COI) and 18S ribosomal DNA. We gathered sequences from temperate (COI n=37; 18S n=31) and polar (COI n=21; 18S n=22) species and then combined them with sequences retrieved from GenBank (COI n=141; 18S n=91) in order to construct phylogenies using all available sequences. We found broad taxonomic diversity within the Nudibranchia of the Ross Sea and recovered reciprocally monophyletic clades of Anthobranchia and Cladobranchia as reported in previous molecular work. Estimates of divergence times of SO lineages from temperate taxa were calculated in three ways, with Bayesian branch lengths and using two molecular clock models implemented using BEAST v1.4.8, a program that jointly infers divergence times as well as phylogenetic relationships among taxa. The COI and 18S tree topologies both show 15 lineages (all >23% divergent at COI from the nearest sequence in the tree) of Antarctic nudibranchs. Seven of these 15 SO lineages contain a single taxon whose closest relative in the phylogeny is a temperate species, while the other eight are contained in three separate clades of SO lineages. This suggests radiation within

the SO over the last 25-60 Mya, coinciding with glacial disturbance of the benthos and the initiation of the Antarctic Circumpolar Current.

## DEDICATION

This work is dedicated to my family. I am the person I am today because of the love and guidance my family has and continues to give me throughout my life. That being said, I could not have completed this project without the unconditional love and support of my beautiful fiancée Jen Gentry. She is always there when I need her with an ear, a shoulder, or a smile. I am so proud of her and who she encourages me to be.

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## INTRODUCTION

The Southern Ocean (SO) is a unique and isolated environment with extreme and constant cold (Clarke, 1983). The SO contains distinctive species assemblages (Aronson *et al.*, 1997; Aronson & Blake, 2001; Gili *et al.*, 2006; Portner, 2006) exhibit unusual biological phenomena. These phenomena include polar gigantism (Chapelle & Peck, 1999; Mikhalevich, 2004; Woods & Moran, 2008; Woods *et al.*, 2009), high endemism (Griffiths *et al.*, 2009), and higher than expected species richness (Arntz & Rios, 1999; Clarke & Johnston, 2003). One explanation for these unique attributes associated with the SO is the hypothesis of faunal isolation within the SO, where oceanographic conditions act as a barrier to dispersal and migration of marine organisms (Dell, 1972; Patarnello *et al.*, 1996; Schrödl, 1999; Hunter & Halanych, 2008; Thornhill *et al.*, 2008). In the traditional view, the isolation of the SO contributes to the uniqueness of SO assemblages through local radiation and adaptation. These processes also may underlie the high endemism that is characteristic of SO fauna.

The Antarctic Circumpolar Current (ACC) runs clockwise around Antarctica and separates the SO from the warmer waters of the Atlantic and Pacific Oceans (Clarke *et al.*, 2005). The ACC moves more water than any other current in the world (Barker & Thomas, 2004). These oceanographic conditions create a thermocline of 3-4°C between the temperate waters and the polar waters of the SO (Gordon *et al.*, 1977; Hunter & Halanych, 2008). The ACC acts as a physical barrier

to water exchange between oceans, and therefore both active and passive dispersal of marine organisms across the current is hindered because the flow of water extends to the seabed in most places along its circumpolar path (Barker & Thomas, 2004). Both the isolation and extreme cold of the SO are attributed to the oceanographic conditions in the south polar region (Clarke, 1983).

The initiation of the ACC is dated between 24 and 41 Mya, concurrent with the estimated age of the opening of the Drake Passage between South America and Antarctica (Lawver & Gahagan, 2003; Pfuhl & McCave, 2005; Scher & Martin, 2006; Wilson *et al.*, 2007). By 22-25 Mya there was deep water (>1000m) in the Drake Passage between South America and Antarctica (Kennett, 1982), which was one necessary element in the establishment the ACC (Clarke *et al.*, 2005). The SO has been <5°C for at least the last 10-14 MY (Sidell, 2000), and likely for most of the last 34 MY (Zachos *et al.*, 2001). Today, nearshore SO waters average -1.8°C with very little (up to +1.5°C in summer) seasonal fluctuation even as far north as the Antarctic Peninsula (Sidell, 2000).

The SO nearshore and shelf are highly disturbed environments, and have been so over the last 65 million years (Barnes, 1999; Thatje *et al.*, 2005 & 2008). Both large-scale and small-scale disturbances may have contributed to species diversity. The massive glaciations of the Cenozoic (65 Mya - present) are thought to have completely ploughed shelf communities and made large areas of the benthic environment uninhabitable in the SO (Gutt, 2001; Thatje *et al.*, 2005; Smale & Barnes, 2008). While this periodic disturbance is likely to have lowered shelf

diversity during glacials, those species that could retreat to deeper habitats or other sheltered areas might have survived to recolonize the shelf after glacial retreat (Thatje *et al.*, 2005). Glacially driven cycles of retreat and re-emergence may have caused vicariant events that fragmented populations and promoted speciation through temporal isolation during interglacials (Clarke & Crame, 1992; Barnes, 1999; Clarke *et al*, 2004; Held & Wägele, 2005; Thatje *et al.*, 2005 Barnes *et al.*, 2006). Small-scale disturbance in the form of iceberg scour may also have served as vicariant events, dividing populations and providing open niches for new species.

The latitudinal gradient hypothesis (Wallace, 1878; Pianka, 1966; Weir & Schluter, 2007) predicts that more species will be found in tropical habitats than in polar ones. In contrast to this prediction, however, Arntz and Rios (1999) demonstrated an increase in species richness from the Magellanic region of South America towards the Antarctic; thus, the SO fails to fit this pattern. Gray (2001) suggested that the barriers between the SO and the surrounding waters might have increased the rate of allopatric speciation within the SO relative to more temperate waters, which may partially explain why there are more species in the SO than would be expected if species diversity decreases with latitude (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000; Gray, 2001; Clarke & Johnston, 2003; Near *et al.*, 2003; Near *et al.*, 2004; Allcock, 2005; Wilson *et al.*, 2007). Overall, the high species richness in the SO is thought to be due to a combination of isolation by the ACC and speciation stemming from disturbance and the isolating effect of the current (Held &

Wägele, 2005; Thatje *et al.*, 2005, 2008; Wilson *et al.*, 2007, 2009; Mahon *et al.*, 2008).

The paradigm of isolation within the SO grew from the perception that the ACC formed a strong barrier to dispersal of marine organisms (Dell, 1972; Patarnello *et al.*, 1996; Schrödl, 1999; Hunter & Halanych, 2008; Thornhill *et al.*, 2008). While the ACC clearly does act as a barrier to dispersal for some groups (Patarnello *et al.*, 1996; Hunter & Halanych, 2008; Thornhill *et al.*, 2008), recent studies have shown that there is in fact some dispersal across the ACC in certain taxa. This dispersal has been found both between South America and Antarctica as well as between Sub-Antarctic islands and Antarctica (Page & Linse, 2002; Near *et al.*, 2004; Clarke *et al.*, 2005; Barnes *et al.*, 2006; Ingels *et al.*, 2006; Wilson *et al.*, 2007; Barnes & Griffiths, 2008). While not all taxonomic groups in the SO share the same biogeographic history, these exceptions to the traditional views are rare (Griffiths *et al.*, 2009). Although the paradigm of biological isolation of the SO is no longer absolute (Clarke *et al.*, 2005; Wilson *et al.*, 2009), it is clear that the SO fauna has high endemism; >50% of SO species are endemic to that ocean (Griffiths *et al.*, 2009; amphipods- Jażdżewski *et al.*, 1991; foraminifers- Mikhalevich, 2004; bivalves and shelled gastropods- Linse *et al.*, 2006; cephalopods- Collins & Rodhouse, 2006; anemones- Rodríguez *et al.*, 2007; bryozoans- Barnes & Griffiths, 2008; pycnogonids- Munilla & Membrives, 2009). While some taxa may be able to disperse across the ACC, the radiation, isolation, and cold in the SO maintain the high endemism.

The phylogenetic affinities of only a few SO taxa, e.g. the notothenioid fish (Bargelloni *et al.*, 2000), have been examined in detail; in these few taxa, molecular phylogenetic studies indicate a high degree of endemism and speciation within the SO. The now diverse SO clade of notothenioid fish is thought to have split from a single sub-Antarctic common ancestor 27 Mya (Bargelloni *et al.*, 2000); similarly, the common ancestor of all SO krill has been estimated to have split from a temperate ancestor 20 Mya (Patarnello *et al.*, 1996). The initiation of these radiation events has been attributed to the emergence of the ACC, which occurred around the same time (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000; Lawver & Gahagan, 2003). This hypothesis of radiation within the SO following a single colonization event has become the standard view of how the diversity of the SO came about, though molecular evidence is lacking for most SO taxa (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000; Strugnell *et al.*, 2008). In the fauna that have been examined using molecular methods, colonization of the SO appears to be a rare event; modern diversity appears to be due to lineage expansion within the SO after single colonizations (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000). One study that does not fit this paradigm has demonstrated that octopus lineages that emerged within the SO octopus fauna were the ancestors of the current Southern Hemisphere deep-sea fauna (Strugnell *et al.*, 2008). The timing of this octopus radiation matches those of the notothenioid fish and krill (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000), yet in this case, the octopus lineages are moving out of the SO into the deep sea at the initiation of the ACC (33 Mya, Strugnell *et al.*, 2008). This supports the

theory of SO radiation, but does not provide information on the origins of the SO octopus fauna other than demonstrating that it is older than 33 Mya (Strugnell *et al.*, 2008).

This study focuses on SO members of the Nudibranchia (Opisthobranchia, Gastropoda, Mollusca), which are shell-less gastropods. The nudibranchs contain >3,000 species, are globally distributed, and are divided into two main clades, the Anthobranchia (Minichev, 1970) and the Cladobranchia (Willan & Morton, 1984). The Anthobranchia are generally more dorso-ventrally flat and have a posterior branchial plume that functions as an external gill, while the Cladobranchia lack the plume and have a branched gut and a dorsum usually covered with cerata (Wägele & Willan, 2000; Schrödl *et al.*, 2001). These two groups are supported both by differences in body structure as well with previous molecular work (Minichev, 1970; Willan & Morton, 1984; Wollscheid & Wägele, 1999; Wollscheid-Lengeling *et al.*, 2001; Grande *et al.*, 2004b; Vonnemann, *et al.*, 2005). Because nudibranchs are soft-bodied, they have no fossil record (Wägele, 2004); therefore, molecular methods are currently the best way to address historical questions of diversity, divergence, and biogeography in this group. Compared to temperate and tropical Nudibranchia, the taxonomy and diversity of the SO nudibranch fauna is poorly known. To the best of our knowledge, also, patterns of endemism in the SO Nudibranchia remain unexplored.

The taxonomy of SO nudibranchs is poorly understood for several reasons. First, many species are described from only a few specimens that may not represent

the morphological variation with a species (Wägele 1987a, 1989b & 1990a), and this may lead to overestimates of species richness. Second, much of the sampling of nudibranchs has been through deeper (often >100m) trawling, meaning the shallow benthic community, which is likely more diverse, has been poorly sampled (Wägele, 1990b & 1991; Cattaneo-Vietti, 1991). Third, nudibranchs in general and SO nudibranchs in particular have a complicated nomenclatural history with many revisions and redescriptions of taxa (Wägele, 1987b, 1989a, 1989b, 1990a, 1990b, & 1991; Cattaneo-Vietti, 1991; Wägele *et al.*, 1995; Schrödl, 2000). This is at least partly due to the fact that nudibranchs as a group exhibit high levels of homoplasy, making it difficult to use morphological characters to define taxa (Gosliner & Ghiselin, 1984).

In order to better understand nudibranch diversity and phylogeography of the SO, we sampled the nearshore fauna of the Ross Sea, Antarctica, and used molecular phylogenetics to assess the number of lineages present and estimate their taxonomic affinities with temperate nudibranchs. Gaining insight into the evolutionary history of the Nudibranchia in the SO will help clarify lineage diversity and divergence as well as shed further light on the paradigm of SO radiation.

## METHODS

*Sampling*

Polar nudibranch specimens were collected by SCUBA in the Ross Sea, Antarctica in waters surrounding McMurdo Station at depths of 10-40 meters (October – December 2006 and 2007). Temperate specimens were collected from the San Juan Islands in Puget Sound, WA, in the eastern Pacific by SCUBA (June/July 2006 and 2007). The diversity of temperate nudibranchs available around the Friday Harbor Laboratories (FHL) provided an accessible collection of taxa that had yet to be examined genetically. All specimens collected were photographed live and were then preserved in 95% ethanol. An additional eight samples of temperate New Zealand nudibranchs, collected intertidally near Auckland, were provided to us by the Auckland War Memorial Museum in Auckland, NZ. Selected samples representing individual nominal species or morphotypes were sequenced (n=47 temperate and n=173 Antarctic) in order to maximize diversity in the dataset. Many SO specimens that were sequenced aligned as duplicate representatives of taxa. Once duplicate sequences were removed, temperate (COI n=37; 18S n=31) and polar (COI n=21; 18S n=22) sequences were combined with sequences from GenBank (Benson *et al.*, 2008) (COI n=141; 18S n=91) to construct a phylogeny using our samples as well as published sequences.

*DNA extraction, PCR, and sequencing*

Tissue samples were extracted using Qiagen DNeasy® Tissue Kits according to the manufacturer's instructions. A 712-bp fragment of mitochondrial cytochrome-c oxidase I (COI) DNA was amplified using nudibranch specific primers developed by the authors (Forward: OpCOIf – 5'-CGTCTTTTTAGGTATGTGATGTGG-3' & Reverse: OpCOIr1 – 5'-CAGCAGGATCAAAGAANCTDG-3'). The COI fragment was amplified through PCR (25µl reactions) using the following cycling conditions: 94°C for 60s; 40 cycles of denaturation at 94°C for 60s, annealing at 40-51°C for 60-90s, extension at 68-70°C for 2 min; final extension of 68-70°C for 10 mins. A 2445-bp fragment of 18S rDNA was amplified (50µl reactions) using primers 18A1 and 1800 (Wollscheid-Lengeling *et al.*, 2001) with conditions: 94°C for 1-2 mins; 40 cycles of denaturation at 94°C for 30s, annealing at 42-52°C for 50-90s, extension at 68-72°C for 2.5 mins; final extension of 68-72°C for 10 mins. In the 18S rDNA amplification reactions dimethyl sulfoxide (DMSO) was included (2µl/50µl reaction) to inhibit the formation of secondary DNA structure. Additionally, Triton-X 100 (Sigma-Aldrich) diluted to 10% was added (0.5µl/50µl reaction) to improve amplification of some 18S fragments. PCR products from both fragments were amplified using a DNA Engine DYAD peltier thermal cycler (MJ Research), visualized through gel electrophoresis, and gel extracted using commercial kits (Qiagen-QIAquick gel extraction & Macherey-Nagel NuceloSpin Extract II) according to the manufacturer's instructions. Purified PCR products were directly sequenced for both COI and 18S

by the Clemson University Genomics Institute. COI was sequenced in both directions using the PCR primers. The 18S fragment was sequenced in pieces and in both directions using the PCR primers plus two internal primers 18SinFor (5'-CAAGYCTGGTGCCAGCAGC-3') and 18SinRev (5'-TGGTGCCCTTCCGTCAAT-3'). Some 18S fragments required the design of clade specific primers for sequencing. These primer sequences are available on request.

*Sequence Alignment*

Sequencing reads were configured and edited using Sequencher 4.2 (Gene Codes) and the resulting sequences were aligned using ClustalW2 (Larkin *et al.*, 2007) under the default settings at the European Bioinformatics Institute web server (http://www.ebi.ac.uk/Tools/clustalw2). The subsequent alignments were edited by eye in Se-Al 2.0va11 (Rambaut, 2002). For the COI dataset, an additional 141 sequences, obtained from GenBank, were added to the 58 lineages sequenced in this study so that all alignments included 199 sequences. For 18S, 91 sequences from GenBank were added to 22 polar and 31 temperate sequences to construct the complete dataset (n=144 sequences). Sequences from GenBank with accession numbers are included in Appendix A. All lineages identified in this study are deposited in GenBank under accessions GQ292022-GQ292079 for COI and GQ326860-GQ326912 for 18S and are presented in Appendix A.

*Phylogenetic Analyses*

Each dataset was evaluated with ModelTest 3.0 (Posada & Crandall, 1998; Posada & Buckley, 2004) in PAUP* 4.0 (Swofford, 2003) under AIC to determine the best model of sequence evolution using the ModelTest Server 1.0 (Posada, 2006). The best fitting model for the COI dataset was the general-time-reversible (GTR) model with gamma-distributed rate heterogeneity among sites (Γ) and a proportion of invariant sites (I); the 18S dataset best fit the GTR+Γ model. Phylogenetic analyses were carried out by Bayesian inference in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For each COI and 18S we ran MrBayes for $5x10^6$ generations with trees sampled every 100 generations. The first $10^4$ trees were discarded as burn-in. A sacoglossan, *Thuridilla hopei* (GenBank accession number: AF249810), was the outgroup for the COI analyses and the anaspidian *Aplysia extraordinaria* (AF249193) served as the outgroup for 18S.

*Divergence Times*

The 199-lineage COI tree was used to estimate divergence times of Antarctic lineages from temperate taxa. Sequence divergence times for the most recent temperate common ancestors (tMRCAs) of Antarctic lineages were calculated using three methods. First, we used an estimate of divergence time based on sequence evolution rates from MrBayes calculated as branch lengths. Sequence divergences

as branch lengths were obtained from the MrBayes analysis and these data, matched with the presented topology, provided measures of divergence between sequences of interest. This method can give a rough approximation of divergence times between lineages or time to the most recent common ancestor. The two additional calculations using the COI dataset were analyzed using an uncorrelated lognormal relaxed molecular clock (RC) model and a strict clock (SC) model implemented in BEAST v1.4.8. This program allows more sophisticated estimates of tMRCA for the SO lineages because of the ability to jointly estimate topologies in coordination with divergence time and estimate across a set of highly likely trees (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). Unlike the SC method, the RC model allows substitution rates to vary across branches in the tree and permits more accurate estimates of divergence times (Ho *et al.*, 2005a; Drummond *et al.*, 2006).

BEAST was run under the GTR+I+Γ nucleotide model with a Yule prior on branching rates and the ingroup constrained as monophyletic (Drummond *et al.*, 2006). Each analysis was run for $4x10^8$ steps with 10% discarded as burn-in. All BEAST runs were repeated to verify consistency and we used a Bayes Factor (BF) comparison of marginal likelihood surfaces performed in Tracer v1.4.1 to determine the best model (RC or SC) to estimate tMRCAs for the SO lineages (Suchard *et al.*, 2001). The degree of support for the BF test comparison was interpreted according to Jeffreys (1961).

Three uniform calibration priors were implemented in BEAST based on biogeographic time points. Fossil calibration points are not available for

nudibranchs; however, the dates of separation of other major ocean basins can provide some constraints on splits in the tree. A transisthmian geminate species clade containing three *Tambja* taxa was constrained at >3 Mya (*T. ceutae* – E. Atlantic - AY345038, Grande *et al.*, 2004a; *T. eliora* – E. Pacific - DQ230998, and *T. morosa* – W. Pacific - DQ230997, Pola *et al.*, 2006). Two trans-arctic clades were used as the other biogeographic calibrations: A *Cadlina* clade was bound >3 Mya (*C. flavomaculata* – E. Pacific - EF535109, Turner & Wilson, 2008; *C. laevis* – E. Atlantic - AY345034, Grande *et al.*, 2004b; *C. luteomarginata* – W. Atlantic - AF249803, Wollscheid-Lengeling *et al.*, 2001) and two sequences of *Aeolidia papillosa* were constrained to be >3 Mya (E. Atlantic - AY345028, Grande *et al.*, 2004a; E. Pacific - this study). The combination of biogeographic events and the incorporation of fossil-based divergence rates from other molluscs provide the best available calibrations for our estimates.

In addition to calibrations based on biogeographic events, we also incorporated fossil-based substitution rates from other molluscs to estimate tMRCAs for our data. Estimates of COI divergence rates for molluscs range from 0.4%-3.1%/MY in the literature (Hellberg & Vacquier, 1999; Marko, 2002; Frey & Vermeij, 2008; Lessios, 2008). We omitted the lowest rate, 0.4%/MY, from consideration because it was estimated without attention to fossils or correction for saturation (Frey & Vermeij, 2008). We chose a rate of 1%/MY for COI sequence divergence based on a fossil-calibrated bivalve mollusc phylogeny of geminate species pairs from the Panamanian isthmus (Marko, 2002). Frey and Vermeij

(2008) also suggest using a rate approximately 1%/MY in favor of faster rates or slower rates. The rationale for this choice of rate involves both the nature of mitochondrial DNA as well as mollusc-specific phylogenetics and fossil congruence. While other rates are of course possible, we chose 1%/MY for several reasons. First, Ho *et al.* (2005b) suggest that rates between 0.5% and 1.5%/MY are most applicable, in general, to protein-coding mitochondrial DNA such as COI. Second, studies estimating molluscan rates derived from fossils and cross-validated with phylogenies suggest using 1%/MY as a divergence rate (Marko, 2002; Frey & Vermeij, 2008). Third, the published sequence divergence rates above 2%/MY are faster than the highest estimates from more thorough studies (1.2%/MY – Marko, 2002; 1.6%/MY – Frey & Vermeij, 2008). In order to address the assortment of fossil-calibrated divergence rates in the literature, we ran three RC Beast analyses using different rates of divergence for COI. We examined a low rate (0.7%/MY-Marko, 2002), our chosen rate of 1%/MY rate (Frey & Vermeij, 2008), and the mean value calculated from published geminate rates (1.93%/MY - Hellberg & Vacquier, 1999; Marko, 2002; Williams & Reid, 2004; Frey & Vermeij, 2008; Lessios, 2008).

## RESULTS

### *Identified Lineages*

Our dataset includes newly sequenced COI and 18S sequences for 34 temperate nudibranch species (Appendix A). One temperate COI lineage is from South Carolina, USA (Appendix A) and eight are from New Zealand (Auckland, NZ). The remaining 26 temperate species are from the San Juan Islands, WA, USA. Our dataset also includes fifteen independent lineages of nudibranchs from the Southern Ocean, eight of which cannot be readily assigned to described species and thus may represent undescribed taxa. One unidentified SO lineage (Lei – Table 1) was characterized only from egg mass tissue samples and no adult specimens were collected that match the egg mass sequence data.

### *Phylogenetic Analyses & SO Radiation*

The 712-bp COI tree (Fig. 1) shows the Nudibranchia are a monophyletic group (posterior probability; PP=1.00) containing two clades, the Anthobranchia (PP=0.32) and the Cladobranchia (PP=0.71), as reported in previous molecular work on Nudibranchia (Wollscheid & Wägele, 1999; Wollscheid-Lengeling *et al.*, 2001; Grande *et al.*, 2004b). The tree contains 15 distinct Southern Ocean lineages (Table 1) that are each >20% divergent in COI sequence from their closest relative

in the tree. The posterior probabilities of these lineages for both COI (PP=0.17-1.00) and 18S (PP=0.64-1.00) are reported in Figure 1. The 15 SO lineages are grouped into ten independent clades. Seven of these ten clades contain a single SO lineage, two contain two lineages (Fig. 2a&b), and one contains four SO lineages (Fig. 2c).

The largest clade of SO taxa contains four discrete (>23% pairwise sequence divergences), morphologically-distinct lineages (Fig. 2c & Fig. 4). The second clade of SO taxa, pairs two lineages (Lei and Unknown D) into a single clade (Fig. 2b), and the third also groups two SO taxa (*Notaeolidia depressa* and *Tritoniella belli)* into a monophyletic clade (Fig. 2a).

The topology of the 2445-bp 18S tree is comparable to that of the COI tree with the Nudibranchia as a monophyletic group (PP=1.00) containing two clades, the Anthobranchia (PP=0.81) and the Cladobranchia (PP=0.99). The 18S tree also supports the identification of all 15 SO lineages and the three clades of SO radiation (Fig. 1). There are some minor topological differences, but these do not affect the identification of the SO lineages and likely arise from the differing gene histories of COI and 18S. The three multispecies SO clades are shown (Fig. 2) with COI sequence divergences mapped onto the branches and divergence time (using RC analysis) at the taxonomic splits.

*Divergence Times*

When the 1%/MY COI molecular clock (Marko, 2002) is applied to the fixed topology inferred with MrBayes, the ten SO clades or lineages share common ancestry with temperate taxa between 34.1-70.1 Mya (Fig. 3, mean tMRCA= 55.98 Mya). All tMRCAs presented in this study refer to divergences between SO lineages and their most recent common ancestor with a temperate (non-SO) taxon in the tree. When we used the Bayes Factor (BF) analysis (Table 2) to compare the fit of the SC and RC models to our dataset, the BF value was $7.7x10^{22}$ in support of the RC. A BF value >100 gives decisive support for the lognormal relaxed clock model over the strict clock model (Jeffreys, 1961; Suchard *et al.*, 2001; Aris-Brosou & Yang, 2002); thus, despite the similarity in the divergence estimates for the three different methods (Fig. 3), the RC model was strongly supported over the SC model. The relaxed clock method estimates splits 28.7-87.2 Mya (Fig. 3, RC mean tMRCA= 61.9 Mya). The tMRCAs for the 15 SO lineages as estimated by the relaxed clock are presented in Table 1. Table 1 shows the tMRCAs for the SO lineages as estimated using our chosen rate (1%/MY) along with two other rates based on the literature (0.7%/MY and 1.93%/MY).

The RC analysis of divergence between SO lineages estimates that the lineages *within* the largest SO clade (Fig. 2c & Fig. 4) diverged 25.3 Mya (Unknown B and Nota light), 26.4 Mya (Previous pair and Unknown E) & 32.8 Mya (Previous three and Nota small). The second example of SO radiation pairs two lineages (Lei

and Unknown D) into a single clade (Fig. 2b) which split 27.0 Mya. In the third SO clade, *Notaeolidia depressa* is estimated to have split from *Tritoniella belli* 59.9 Mya (Fig. 2a).

## DISCUSSION

*Nudibranch Diversity in the SO*

The overall topology of our COI tree, which contains 58 novel sequences, is consistent with previous studies that defined two major clades in the Nudibranchia, the Anthobranchia and the Cladobranchia (Wollscheid & Wägele, 1999; Wollscheid-Lengeling *et al.*, 2001Grande *et al.*, 2004b). The diversity of SO nudibranch lineages in our dataset provides an opportunity to investigate the taxonomic affinities of this SO fauna, and to explore the biogeographic history of the SO nudibranchs. Our COI and 18S trees both show that the SO lineages are spread throughout the Nudibranchia and most (10 out of 15) Antarctic lineages are paired to temperate sister taxa. This suggests at least 10 independent origins of the SO nudibranch fauna.

Even in temperate oceans, sequence data can be isolated and sparse. Despite the existence of a marine lab at Friday Harbor, WA for over 100 years, little sequencing work had been done on the diverse nudibranch fauna of the area. In the SO, very few molecular sequence data are available. Previously, only four SO nudibranchs had 18S or COI sequence data published in GenBank. The description of new species from the SO is not uncommon as much of the Antarctic is still unexplored when compared to temperate and tropical environments, yet descriptions are often based solely on morphologic variation (Cantero, 2009; Dailey,

2009; López-González *et al.*, 2009; Matallanas, 2009) and not the identification of novel lineages using sequence data (Wilson *et al.*, 2007; Turner & Wilson, 2008; Minck *et al.*, 2009). Cryptic speciation has been demonstrated in the SO when using molecular techniques to examine diversity (Held & Wägele, 2005; Wilson *et al.*, 2007; Turner & Wilson, 2008). Understanding the diversity of nudibranch lineages in the SO serves as an important first step in investigating and interpreting SO biogeographic processes.

*Time of Divergence of SO Nudibranchs from Temperate Relatives*

Relaxed clock methods are often found to be a better fit to a dataset when compared with strict clock methods (Yoder & Yang, 2000; Aris-Brosou & Yang, 2002; Brown *et al.*, 2008), possibly because constant rates of sequence evolution (as assumed when using a SC) may be rare when dealing with higher taxonomic levels (Welch & Broham, 2005). There are many studies suggesting that DNA evolution rate heterogeneity is common, even within closely related taxa (Wu & Li, 1985; Britten, 1986; Yoder & Yang, 2000; Aris-Brosou & Yang, 2002; Woolfit & Bromham, 2003; Davies *et al.*, 2004; Drummond *et al.*, 2006; Lanfear *et al.*, 2007). Using the RC model with three different divergence rates, we see a wide range of estimated tMRCAs for SO lineages (Table 1; 0.7%/MY = 39.7-119.9 Mya; 1.0%/MY = 28.7-87.2 Mya; 1.93%/MY = 40.9-116.5 Mya). The breadth of these ranges within each divergence model suggests varied biogeographic histories for different SO lineages

of Nudibranchia, with some being approximately concurrent with the establishment of the ACC and some substantially older.

In a species level analysis of a SO nudibranch, Wilson *et al.* (2009) used a much more rapid rate of 2.4%/MY to estimate divergence time of cryptic taxa within *Doris kerguelenensis* (also in our dataset). This rate, however, was taken from a study (Hellberg & Vacquier, 1999) that calibrated a COI divergence rate based on a transisthmian geminate pair of *Tegula* (Orthogastropoda, Gastropoda, Mollusca) species that, according to the fossil record and its position in a broader snail phylogeny (Hellberg, 1998; Marko, 2002), is much older than the final closure of the Central American Seaway (Marko, 2002), and thus this rate is likely too fast. If Hellberg and Vacquier's (1999) rate is recalculated using *Tegula*'s phylogenetic affinities, the 2.4%/MY rate drops to 0.7%-1.5%/MY (Marko, 2002). Therefore, we feel the 1%/MY rate is more appropriate and most likely to reveal the true evolutionary history of this group.

Molecular evolution rates might be expected to be comparatively slowed in SO ectotherms with longer generation times due to extreme cold temperature (Laird *et al.*, 1969; Bleiweiss, 1998; Gillooly *et al.*, 2005); if that were the case, rates of COI change estimated from tropical taxa (such as our rate of 1%/MY, which comes from Central American taxa) might be inappropriately rapid for polar taxa. However, using the individual branch rate estimates from BEAST, we found no evidence of a substitution rate slowdown in SO lineages relative to warmer-water groups. Estimated substitution rates on terminal temperate branches ranged from 0.75% -

1.38%/MY (average – 1.00%/MY), while the rates on branches of SO lineages were 0.75% - 1.23%/MY (average – 1.03%/MY). In paired comparisons of rates of the 10 SO lineages and their nearest temperate relatives, SO lineages had slower rates than temperate sister taxa in only 4/10 pairs; in the other six paired comparisons, temperate taxa had slower rates of COI change than their polar relatives (Table 3). This is perhaps not surprising given that Lanfear *et al.* (2007) examined over 300 metazoan taxa and 12 different genes (including COI & 18S) and found no correlation between mass-specific basal metabolic rate and substitution rate; thus, the slowed metabolisms of polar taxa might not in fact be expected to reduce rates of COI substitution in Nudibranchia.

Using the divergence dates calculated under the 1%/MY RC method with the COI dataset, we estimated that SO lineages split from their temperate relatives between 28.7 and 87.2 Mya. Most of the estimated divergence dates in our dataset almost certainly predate the true split of SO animals from their closest temperate relatives, because the global sampling of nudibranchs has a strong Northern Hemisphere bias. The deep splits between SO and temperate relatives in our dataset likely reflect overall poor sampling of the nudibranch fauna of the Southern Hemisphere. Only a small proportion of the total number of non-polar nudibranch sequences in our dataset (15/176 COI and 16/119 18S) come from the temperate Southern Hemisphere; it seems highly unlikely, therefore, that the temperate-SO pairings in our dataset represent true sister pairings. Because the closest temperate relatives of SO lineages are probably missing from our analyses, the true divergence

times for SO lineages from temperate ones are likely considerably more recent than 28.7-87.2 Mya. However, despite this limitation, the phylogenetic spread of SO lineages across distant Nudibranchia clades, suggests multiple moves into the SO by nudibranch taxa. At the very least, there have been both anthobranch and cladobranchs that have moved into the SO.

Based on the apparent recency of one of our estimates, some colonization of the SO may have occurred after the establishment of the ACC. The divergence estimate of note is between *Doto antarctica* (#3 – Table 1) from the SO (~78°S) and *D. columbiana* from Friday Harbor, WA (48°N). COI estimates with 1%/MY place this split at 28.7 Mya, which seems surprising given that these two species were sampled at locations that are nearly 15000 km apart. The SO lineage *D. antarctica* may, therefore, represent an invasion of the SO after the establishment of the ACC by another, Southern Hemisphere member of the genus *Doto* (that is not represented in the molecular dataset). Sequence data from a Southern Hemisphere representative of *Doto* would be very informative. Without these Southern Hemisphere *Doto*, we are unable to confirm that they are the closest relatives to the SO *Doto* in our dataset and the possibility remains that the two *Doto* species in our dataset are potentially antitropical sister species pair set up by migration across the equator (Lindberg, 1991; Hilbish *et al.*, 2000).

*Southern Ocean Radiation*

Recent work suggests that isolation of the SO by the ACC and the unique environmental conditions of the SO promote diversification within Antarctic waters (Bargelloni *et al.*, 2000; Clarke & Johnston, 2003; Near *et al.*, 2003; Near *et al.*, 2004; Allcock, 2005; Wilson *et al.*, 2007). Like many other taxa, nudibranch molluscs show high (71% of species, 45% of genera) endemism in the SO (Table 4, Appendix B), which is consistent with both isolation and a model of frequent lineage radiations within the SO. Our data suggest that three of the SO lineages have radiated since their arrival in the SO (Fig. 2), a pattern similar to those seen in krill (Patarnello *et al.*, 1996) and notothenioid fish (Bargelloni *et al.*, 2000). These radiations likely reflect expansion and speciation of SO lineages after their arrival in the SO (Fig. 2).

Within the three multi-species clades of SO nudibranchs, the dates of radiation roughly span the period from early Cenozoic glaciation (65 Mya – Thatje *et al.*, 2005) to the initiation of the ACC (24-41 Mya – Lawver & Gahagan, 2003; Pfuhl & McCave, 2005; Scher & Martin, 2006; Wilson *et al.*, 2007) (Table 1; 1.0%/MY – 25.3-59.9 Mya). The dates of radiation estimated within two of these clades (25.3-32.8 Mya – Fig. 2b,c) roughly coincide with the initiation of the ACC, consistent with the idea that, as is the case for notothenioids and krill (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000), the isolation of the SO by the ACC may have been a factor driving the radiation of SO nudibranch fauna. The split (Fig. 2a) between *N. depressa* and *T. belli* at 59.9 Mya places the emergence of the oldest pair of SO lineages soon after the

start of severe glaciation in the benthos (beginning ~65 Mya; Thatje *et al.*, 2005) possibly suggesting that large-scale glacial disturbance may have played a role in increasing diversity as hypothesized by Thatje *et al.* (2005). However, these clades could also contain temperate Southern Hemisphere species that we have not sampled; if that were the case, these might not represent true radiations in the SO.

All lineages within the three clades of SO radiation are >20% divergent at COI from the other lineages in their clades. Within one additional nominal species, *Doris kerguelenensis* (Bergh, 1884) (#15 - Table 1), we also see considerable (>10%) COI sequence variation among samples. Wilson *et al.* (2009) also found very high levels of mitochondrial lineage diversification within *D. kerguelenensis*, suggesting cryptic speciation since the initiation of the ACC. Wägele (1990a) made note of the high level of morphological variation in distinguishing characters in this species, yet found no morphological patterns to suggest this variation should merit multiple species. In fact *D. kerguelenensis* (then *Austrodoris* until Valdés, 2002) was synonomized from multiple (~14) nominal species because there were not any consistent patterns of morphological variation (Wägele, 1990a). Our molecular data for this species are similar to Wilson *et al.* (2009), in that we found 12.4% sequence divergence between two lineages within *D. kerguelenensis.* Using our 1%/MY relaxed clock sequence evolution model gives an age estimate of 16.2 Mya for the clade of *D. kerguelenensis* in the COI tree. This may be an example of ongoing cryptic speciation due to population fragmentation caused by periodic glaciation of the SO benthos (Thatje *et al.*, 2005, 2008; Wilson *et al.* 2009).

Both this study and Wilson *et al.* (2009) provide further support for the idea that lineage diversification within the SO Nudibranchia contributes to the current diversity present in this group in the SO. More than half (8 of 15) of the SO lineages (>20% divergent at COI) in the nudibranch COI tree are contained in the three multispecies clades that may have radiated within the SO; thus, the isolation (even if not complete) from temperate waters provided by the ACC and subsequent speciation within the SO appear to be structuring the nudibranch diversity we see today in the McMurdo Sound region of the Ross Sea (Held & Wägele, 2005; Thatje *et al.*, 2005, 2008; Wilson *et al.*, 2007, 2009; Mahon *et al.*, 2008).

*Southern Ocean Nudibranchia*

Another pattern that has emerged from our data is a disparity between representation of the two main nudibranch lineages, Cladobranchia and Anthobranchia in the SO; the Anthobranchia appear to be underrepresented in the SO compared to the surrounding oceans of the Southern Hemisphere. In the temperate waters of the Southern Hemisphere, 63% of named species (266/424) and 55% of genera (71/130) are Anthobranchia (Table 5). Yet, in this study of the SO nudibranch diversity, a much smaller proportion of lineages (3/15, or 20%) are Anthobranchia. This apparent taxonomic bias towards Cladobranchia in the SO is significant under a Chi-squared contingency test in which each monophyletic lineage (n=10) was tested as a single data point ($\chi^2$=11.15, 1 d.f. $p<0.001$). One

potential explanation for the dominance of Cladobranchia in the SO is sampling error based on under-collection of this group. However, because of the evident shortage of anthobranch diversity during two field seasons of SCUBA diving in McMurdo Sound, divers searched intensively for Anthobranchia early on and throughout the collecting process. Quantitative estimates of frequency were not measured during collections (>200 dives over two years), but it seems likely that, at least within diving depths and in the accessible habitats around McMurdo Station, members of the Anthobranchia are substantially less diverse in McMurdo Sound than the Cladobranchia.

Another possible though entirely untested explanation for the scarcity of Anthobranchia in McMurdo Sound is one based on life history differences between these two major groups of nudibranchs. Clark (1975) divided nudibranchs into two ecological types based on successional stage, "exploitists" and "strategists." "Exploitist" nudibranchs have characteristics that correspond with early successional species, such as rapid growth, early maturation, and shorter generation times; these are the taxa that might be expected to occur in highly disturbed habitats. "Strategists" are late successional species that grow slowly and reproduce at later ages and with longer generation times. According to Clark (1975), nudibranch exploitists fall into the Cladobranchia, while strategists fall into the Anthobranchia. Clark hypothesized that cladobranchs in general make better exploitists than anthobranchs because, unlike the anthobranchs, cladobranchs have a highly branched gut that extends into their dorsal cerata. A larger gut surface area

may allow cladobranchs to have increased food assimilation, growth, and metabolism relative to anthobranchs, and to fit into the opportunist niche more easily.

The Antarctic nearshore environment is one of the most highly disturbed environments in the world due to ice scour and periodic glaciation (Dayton & Oliver, 1977; Dayton *et al*., 1994; Thatje *et al*., 2005; Thrush *et al*., 2006). Because of these disturbances, the polar benthos is permanently in a state of change or recovery (Barnes, 1999; Gili *et al.*, 2006). If Antarctic cladobranch nudibranchs indeed tend to have life history characteristics such as rapid growth and early reproduction, they may be more suited to the highly disturbed environment of the Antarctic than the Anthobranchia. Both the paucity of Anthobranchia in the SO and the radiation we see within clades of Antarctic cladobranchs are consistent with this idea. However, more information on the life history of Antarctic species in general, and Southern Hemisphere nudibranchs in particular, is needed to further explore how these traits may, or may not, be related to the diversity, richness, and biogeography of SO marine organisms.

*Conclusions & Implications*

Our data regarding nudibranchs in the Ross Sea, Antarctica define 15 phylogenetically diverse lineages that separated from multiple temperate ancestors over tens of millions of years, suggesting that more than one biogeographic process

or event drove the emergence of the nudibranch fauna. The timing of SO radiations in the Nudibranchia imply that local radiation is acting on similar timescales in these molluscs as in other SO taxa (Patarnello *et al.*, 1996; Bargelloni *et al.*, 2000), and that within-ocean radiation has contributed to the diversity of SO Nudibranchia. On a global scale and across multiple taxa, little phylogenetic work has been done in the Southern Hemisphere compared to the Northern Hemisphere. A recent review places only 15% of all phylogenetic studies in the Southern Hemisphere, and Antarctic taxa constitute < 1% of the phylogenetic literature (Beheregaray, 2008). Moving forward, more sampling needs to be done throughout the SO, so that molecular analyses can be completed with a larger geographic scope than the Ross Sea and the Northern Hemisphere biases in sequence data can be eliminated. With the elevated status of Antarctic research surrounding the International Polar Year 2007-08, there are several large projects ongoing that are actively using molecular techniques in the SO to pursue better understanding of SO biodiversity, taxonomic distribution, and faunal origins and evolution (EBA-Evolution and Biodiversity in the Antarctica; CAML- Census of Antarctic Marine Life; ICEFISH-2007). Increased sampling of the SO will both improve the broad view of SO fauna and permit more extensive and taxonomically specific examinations of cryptic speciation, diversity, population structure, and radiation within the SO (see Wilson *et al.*, 2009).

**TABLES**

**Table 1.** The 15 Southern Ocean lineages (in 10 clades) with their closest relative in the dataset showing COI sequence divergence and estimated divergence. T- closest relative is temperate. A- closest relative is Antarctic. Labels related to other figures. RC is date of divergence between lineage and the listed closest relative in Mya using relaxed clock estimation using three substitution rates (%/MY).

| Label | SO Lineage/Clade | % Div. | Closest Relative | RC- 0.7% (Mya) | RC- 1% (Mya) | RC- 1.93% (Mya) |
|---|---|---|---|---|---|---|
| 11 | *Charcotia granulosa* | 64.2 | T- *Armina californica* | 87.1 | 63.4 | 89.0 |
| | | | | | | |
| 13 | Dorid YB | 62.0 | T- *Chromodoris* clade | 102.6 | 73.8 | 97.0 |
| | | | | | | |
| 14 | Dorid Unknown A | 68.8 | T- *Actinocyclus verrucosus* | 119.9 | 87.2 | 116.5 |
| | | | | | | |
| 3 | *Doto antarctica* | 34.1 | *T- Doto columbiana* | 39.7 | 28.7 | 40.9 |
| | | | | | | |
| 10 | Purple | 60.2 | T- *Eubranchus* clade | 98.0 | 71.1 | 89.2 |
| | | | | | | |
| 12 | *Tritonia challengeriana* | 45.4 | T- *Tritonia* clade | 71.7 | 52.0 | 68.7 |
| | | | | | | |
| 15 | *Doris kerguelenensis* | 70.1 | T- *Archidoris* clade | 105.2 | 76.6 | 100.2 |
| | --within *D. kerguelenensis* | 12.4 | | 22.6 | 16.2 | 18.3 |
| | | | | | | |
| A | *N. depressa/ T. belli* clade | 66.3 | *T- Flabellina* clade | 97.6 | 71.2 | 103.1 |
| 2 | --*Notaeolidia depressa* | 55.6 | A- *T. belli* | 82.6 | 59.9 | 95.4 |
| 1 | --*Tritoniella belli* | 55.6 | A- *N. depressa* | 82.6 | 59.9 | 95.4 |
| | | | | | | |
| B | Unknown D/Lei clade | 43.2 | T- *Eubranchus sanjuanensis* | 59.7 | 43.4 | 59.8 |
| 4 | --Lei | 30.2 | A- Unknown D | 37.2 | 27.0 | 36.3 |
| 5 | --Unknown D | 30.2 | A- Lei | 37.2 | 27.0 | 36.3 |
| | | | | | | |
| C | Nota clade | 45.5 | T- *Eubranchus* clade | 71.6 | 52.0 | 68.1 |
| 7 | --Unknown B | 23.8 | A- Nota Light | 35.3 | 25.3 | 32.4 |
| 8 | --Nota Light | 23.8 | A- Unknown B | 35.3 | 25.3 | 32.4 |
| 6 | --Unknown E | 24.2 | A- 2 previous | 36.6 | 26.4 | 32.9 |
| 9 | --Nota Small | 23.7 | A- 3 previous | 45.4 | 32.8 | 41.0 |

**Table 2.** Bayes Factor (BF) comparison of the two clock methods used in BEAST to estimate divergences between lineages. RC= uncorrelated lognormal relaxed molecular clock and SC= strict molecular clock. ln P =marginal tree likelihood of the model and S.E. = standard error of estimate (using 1000 bootstrap replicates).

| **Model** | **ln P(model → data)** | **S.E.** | **Bayes Factor** | |
|---|---|---|---|---|
| | | | vs. RC | vs. SC |
| RC | -39707.343 | +/- 0.361 | -- | 7.7E+22 |
| SC | -39760.039 | +/- 0.289 | 0 | -- |

**Table 3.** Pairwise COI substitution rate comparisons between SO lineages and their nearest temperate relative in the COI tree estimated using the RC Beast analysis. SO rates that are slower than temperate rates are shown in bold.

| **SO Name** | **Temperate Relative** | **Temp. Rate** | **SO Rate** |
|---|---|---|---|
| *Charcotia granulosa* | T- *Armina californica* | 0.592% | 0.604% |
| Dorid YB | T- *Chromodoris* clade | 0.499% | 0.518% |
| Dorid Unknown A | T- *Actinocyclus verrucosus* | 0.521% | **0.479%** |
| *Doto antarctica* | *T- Doto columbiana* | 0.536% | **0.490%** |
| Purple | T- *Eubranchus* clade | 0.498% | **0.493%** |
| *Tritonia challengeriana* | T- *Tritonia* clade | 0.486% | 0.578% |
| *Doris kerguelenensis* | T- *Archidoris* clade | 0.506% | **0.499%** |
| *N. depressa/ T. belli* clade | *T- Flabellina* clade | 0.493% | 0.537% |
| Unknown D/Lei clade | T- *Eubranchus sanjuanensis* | 0.468% | 0.557% |
| Nota clade | T- *Eubranchus* clade | 0.498% | 0.507% |

**Table 4.** Percent endemism of temperate nudibranchs by Southern Hemisphere region at specific and generic levels. These data are compiled from published range data and presented in its entirety in Appendix B.

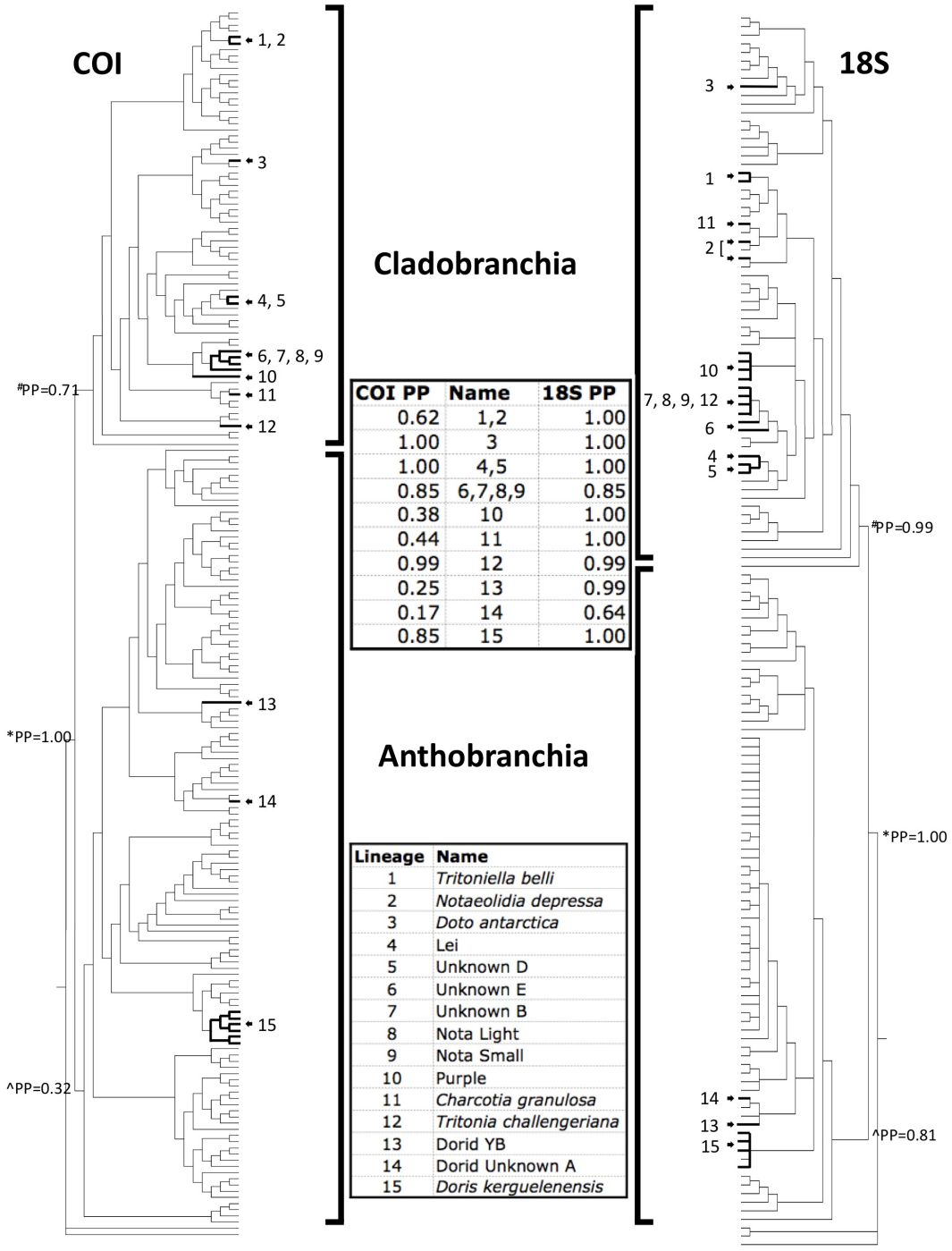
| **Species** | | | |
|---|---|---|---|
| **Region** | **Endemics** | **Total Species** | **Endemic %** |
| S. Africa | 160 | 204 | 78.4 |
| S. America | 38 | 44 | 86.4 |
| Antarctica | 12 | 17 | 70.6 |
| Austr. & NZ | 133 | 198 | 67.2 |
| | | | |
| **Genera** | | | |
| **Region** | **Endemics** | **Total Genera** | **Endemic %** |
| S. Africa | 26 | 88 | 29.6 |
| S. America | 5 | 28 | 17.9 |
| Antarctica | 5 | 11 | 45.5 |
| Austr. & NZ | 25 | 85 | 29.4 |

**Table 5.** Numbers of anthobranch species and genera from dataset gathered in Appendix B compared to the findings in the SO. Numbers in parentheses represent the total number of species, genera, or lineages in the dataset.

| **Temperate S. Hemisphere** | |
|---|---|
| Anthobranch species | 266 |
| %Anthobranchia (424) | 62.7% |
| | |
| Anthobranch genera | 71 |
| % Anthobranchia (130) | 54.6% |
| | |
| | |
| **Southern Ocean** | |
| Anthobranch lineages | 3 |
| % Anthobranchia (15) | 20.0% |

**FIGURES**

**Figure 1.**




| COI PP | Name | 18S PP |
|---|---|---|
| 0.62 | 1,2 | 1.00 |
| 1.00 | 3 | 1.00 |
| 1.00 | 4,5 | 1.00 |
| 0.85 | 6,7,8,9 | 0.85 |
| 0.38 | 10 | 1.00 |
| 0.44 | 11 | 1.00 |
| 0.99 | 12 | 0.99 |
| 0.25 | 13 | 0.99 |
| 0.17 | 14 | 0.64 |
| 0.85 | 15 | 1.00 |

| Lineage | Name |
|---|---|
| 1 | *Tritoniella belli* |
| 2 | *Notaeolidia depressa* |
| 3 | *Doto antarctica* |
| 4 | Lei |
| 5 | Unknown D |
| 6 | Unknown E |
| 7 | Unknown B |
| 8 | Nota Light |
| 9 | Nota Small |
| 10 | Purple |
| 11 | *Charcotia granulosa* |
| 12 | *Tritonia challengeriana* |
| 13 | Dorid YB |
| 14 | Dorid Unknown A |
| 15 | *Doris kerguelenensis* |

**Figure 2.**

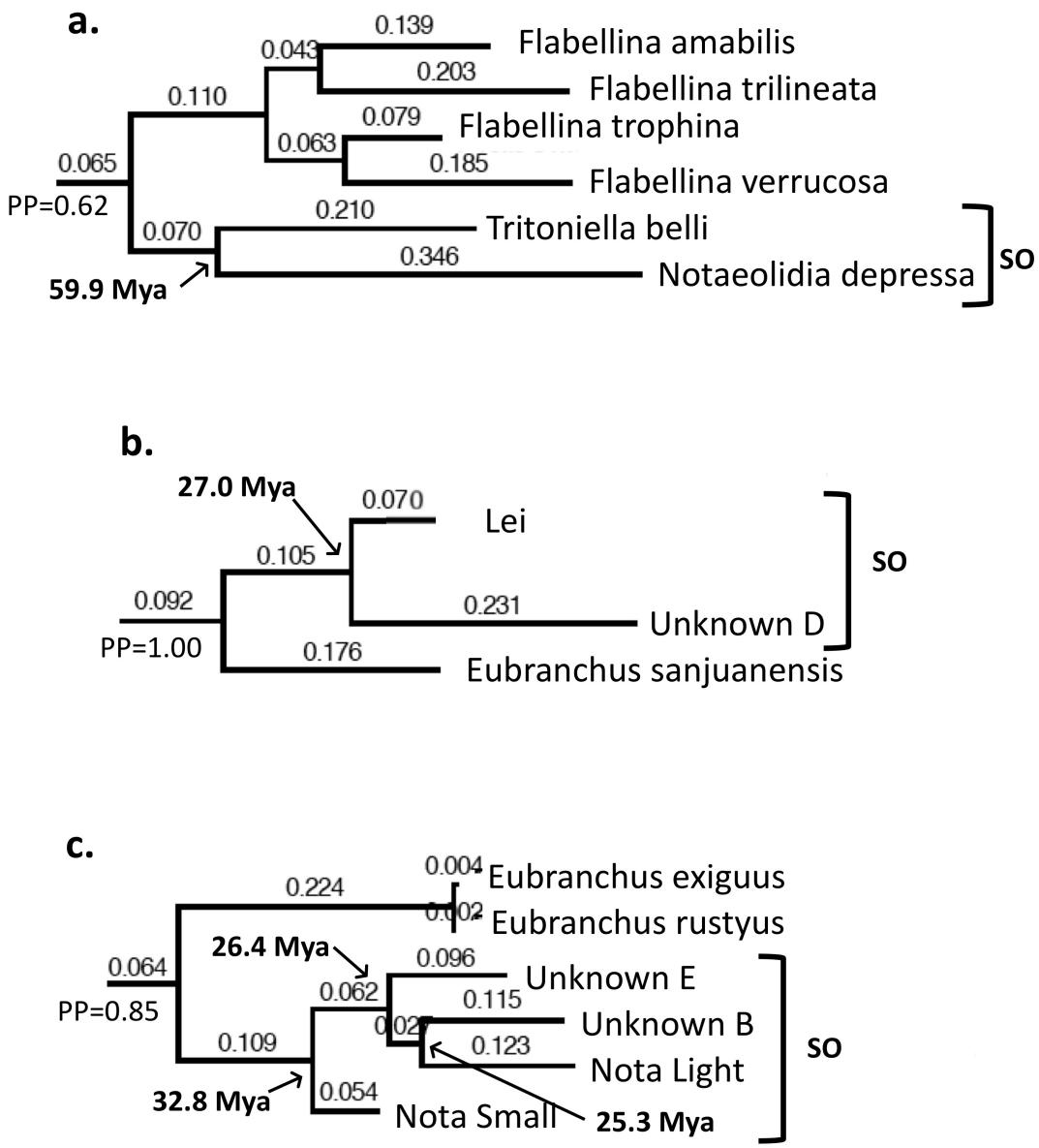

a.
0.139
Flabellina amabilis
0.043
0.203
Flabellina trilineata
0.110
0.079
Flabellina trophina
0.063
0.065
0.185
Flabellina verrucosa
PP=0.62
0.210
Tritoniella belli
0.070
0.346
Notaeolidia depressa
SO
59.9 Mya
b.
27.0 Mya
0.070
Lei
0.105
SO
0.092
0.231
Unknown D
PP=1.00
0.176
Eubranchus sanjuanensis
c.
0.004
Eubranchus exiguus
0.224
0.002
Eubranchus rustyus
26.4 Mya
0.096
0.064
Unknown E
0.062
0.115
PP=0.85
Unknown B
0.109
0.123
Nota Light
SO
0.054
32.8 Mya
Nota Small
25.3 Mya

**Figure 3.**

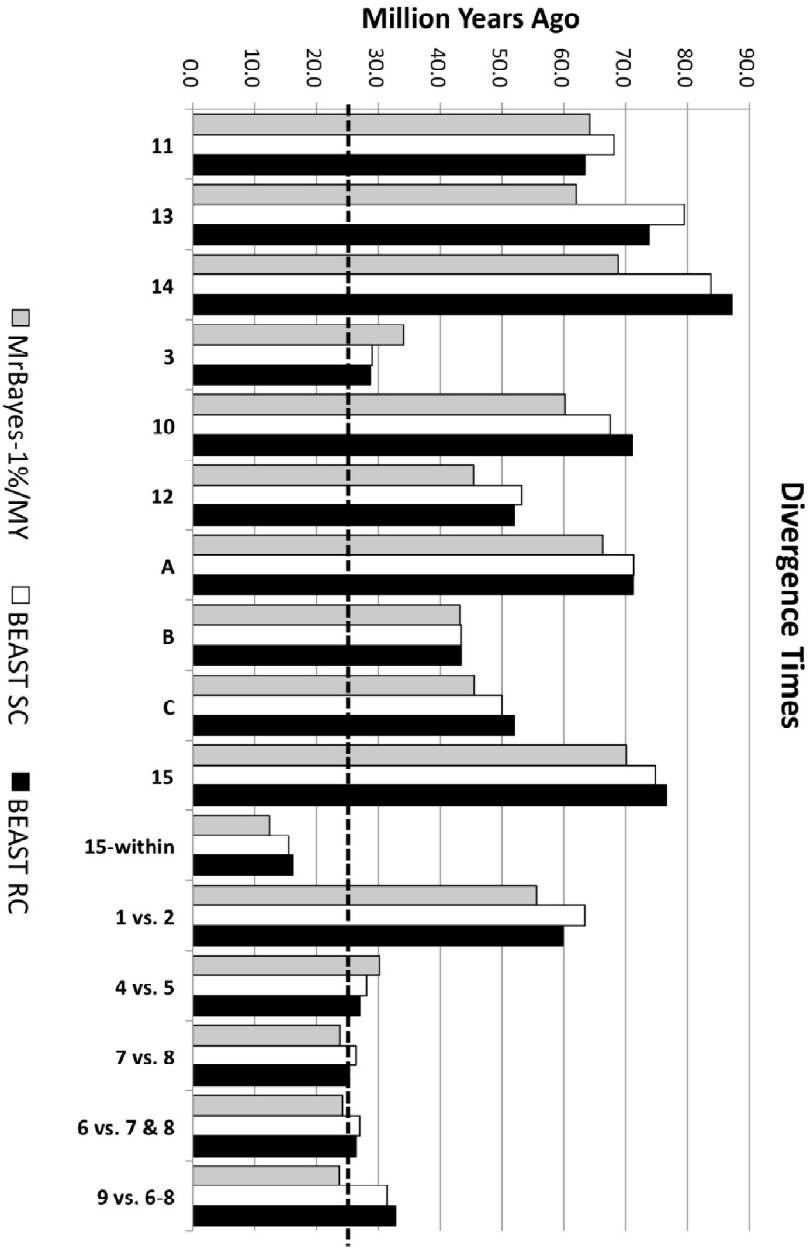
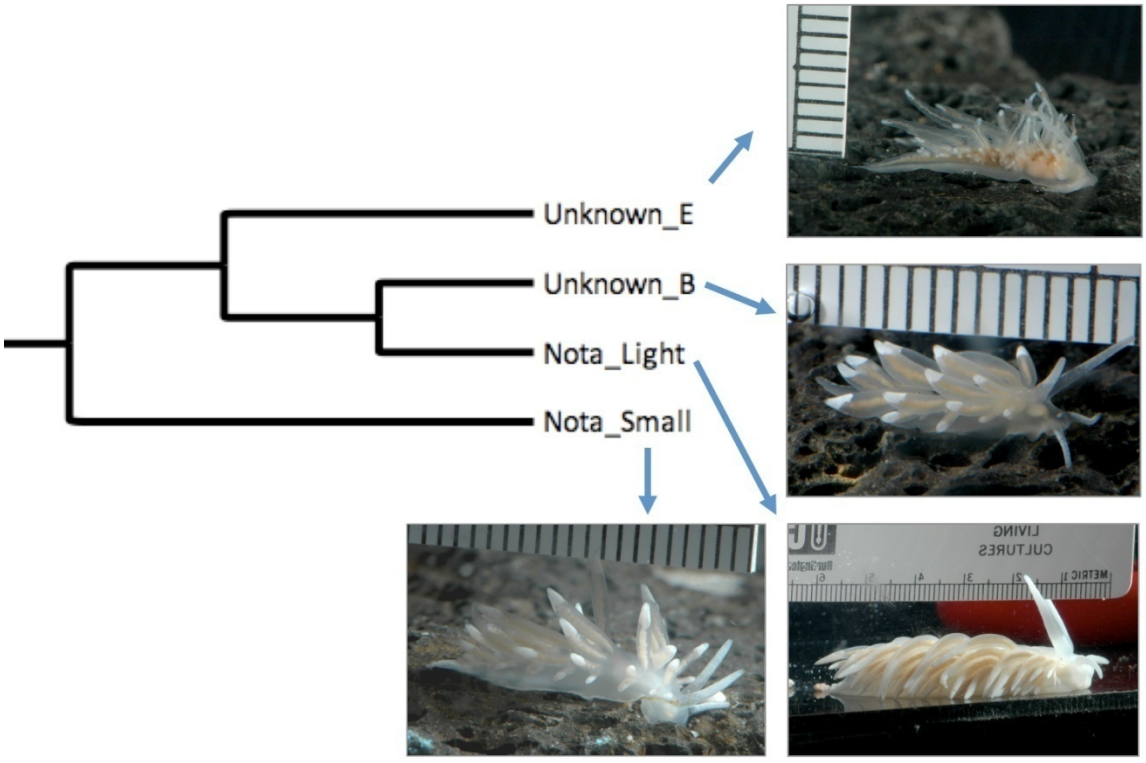

Million Years Ago
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
11
13
14
3
10
12
A
B
C
15
15-within
1 vs. 2
4 vs. 5
7 vs. 8
6 vs. 7 & 8
9 vs. 6-8
MrBayes-1%/MY
BEAST SC
BEAST RC
Divergence Times

**Figure 4.**



## FIGURE CAPTIONS

**Figure 1.** COI and 18S tree topologies demonstrating the 10 clades and 15 lineages of SO nudibranchs identified in this study. Bold branches note SO lineages. Numbers mark individual SO lineages (identified in Table 1) and are maintained on both trees. Multiple branches with one number represent multiple sequences identified as one lineage. The upper included table notes posterior probabilities (PP) of nodes between SO lineages and closest temperate relatives. The lower table identifies the numbered lineages. PP labels on nodes refer to Nudibranchia*, Cladobranchia#, and Anthobranchia^ nodes.

**Figure 2.** COI topologies of the three clades demonstrating SO radiations in the Nudibranchia. Brackets denote SO lineages. Numbers above branches are COI branch lengths from MrBayes. PP= Posterior Probability from MrBayes of node representing temperate/SO split. Ages in Mya are RC estimates of divergence times between SO lineage radiations. Letters refer to labels in Table 1.

**Figure 3.** Comparison of the three methods used to estimate divergence times in Mya using COI dataset. Gray bars represent sequence divergences calculated from branch lengths in MrBayes with a 1%/MY (Marko, 2002) strict clock. White bars are a strict clock (SC) implemented in BEAST with a prior substitution rate of 1%/MY. Black bars represent the estimates from an uncorrelated lognormal relaxed clock (RC) model with a 1%/MY prior rate applied in BEAST. The dashed line represents the initiation of the ACC (~25 Mya). Labels are consistent with those in Table 1.

**Figure 4.** Morphological divergence in the four SO lineages indentified through molecular divergence in the largest SO clade. Scale for photos are in mm. Branch lengths are not representative of divergence.

**APPENDICES**

**Appendix A.** Collection sites, GenBank accession numbers and references for all sequences used in this study. Collection sites marked with an * denote those sequences were obtained during this study. Outgroup sequences are noted as (SAC) = sacoglossan (ANA) = anaspidean. References: [1]Thollesson, 2000; [2]Wollscheid & Wägele, 1999; [3]Turner & Wilson, 2008; [4]Grande *et al.*, 2004a&b; [5]Wollscheid-Lengeling *et al.*, 2001; [6]Fahey, 2003; [7]Wägele *et al.*, 2003; [8]Faucci *et al.*, 2007; [9]Passamaneck *et al.*, 2003; [10]Giribet & Wheeler, 2002; [11]Eriksson *et al.*, 2006; [12]Pola *et al.*, 2006; [13]Direct submissions; [#]Samples from the Auckland War Memorial Museum-Auckland, NZ.

| **Taxon** | **COI Collection Site** | **COI** | **18S Collection Site** | **18S** |
|---|---|---|---|---|
| *Acanthodoris brunnea* | USA, Washington* | GQ292045 | USA, Washington* | GQ326876 |
| *Acanthodoris pilosa* | Sweden[1] | AJ223254 | North Sea[2] | AJ224770 |
| *Actinocyclus verrucosus* | Australia, Queensland[3] | EF535108 | -- | -- |
| *Aeolidia papillosa* | Spain, NE Atlantic[4] | AY345028 | -- | -- |
| *Aeolidia papillosa* | USA, Washington* | GQ292049 | -- | -- |
| *Aldisa banyulensis* | Madeira, Portugal[4] | AY345039 | -- | -- |
| *Ancula gibbosa* | Scotland[4] | AY345029 | -- | -- |
| *Aphelodoris luctuosa* | NZ, Auckland[#] | GQ292042 | NZ, Auckland[#] | GQ326867 |
| *Aphelodoris* sp. 1 | -- | -- | NZ, Auckland[#] | GQ326866 |
| *Aphelodoris* sp. 2 | NZ, Auckland[#] | GQ292033 | NZ, Auckland[#] | GQ326868 |
| *Aplysia extraordinaria* (ANA) | Australia, Queensland[5] | AF249823 | Australia, Queensland[5] | AF249193 |
| *Archidoris montereyensis* | USA, Washington* | GQ292041 | USA, Washington* | GQ326870 |
| *Archidoris pseudoargus* | UK, Devonshire[1] | AJ223256 | North Sea[5] | AF249217 |
| *Archidoris wellingtonensis* | NZ, Auckland[#] | GQ292034 | NZ, Auckland[#] | GQ326869 |
| *Ardeadoris egretta* | Indonesia[3] | EF535140 | Indonesia[3] | EF534022 |
| *Armina californica* | USA, Washington* | GQ292055 | USA, Washington* | GQ326884 |
| *Armina lovenii* | North Sea[5] | AF249781 | North Sea[5] | AF249196 |

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|---|---|---|---|---|
| *Asteronotus cespitosus* | Western Australia[6] | AY128126 | -- | -- |
| *Bathydoris clavigera* | Antarctica, Weddell Sea[5] | AF249808 | Antarctica, Weddell Sea[7] | AY165754 |
| *Bornella stellifer* | -- | -- | Australia, Queensland[7] | AY165756 |
| *Cadlina flavomaculata* | USA, California[3] | EF535109 | -- | -- |
| *Cadlina laevis* | Scotland[4] | AY345034 | Scotland[3] | EF534039 |
| *Cadlina luteomarginata* | USA, North Atlantic[5] | AF249803 | USA, North Atlantic[2] | AJ224772 |
| *Cadlinella ornatissima* | -- | -- | Australia, Queensland[3] | EF534030 |
| *Caloria indica* | USA, Hawaii[8] | DQ417325 | -- | -- |
| *Ceratosoma amoena* | -- | -- | Australia, New South Wales[3] | EF534021 |
| *Ceratosoma trilobatum* | Australia, Queensland[3] | EF535142 | Australia, Queensland[3] | EF534025 |
| *Charcotia granulosa* | Antarctica, Ross Sea* | GQ292060 | Antarctica, Ross Sea* | GQ326885 |
| *Chromodoris alternata* | Australia, New South Wales[3] | EF535120 | Australia, New South Wales[3] | EF534031 |
| *Chromodoris ambiguus* | Australia, New South Wales[3] | EF535119 | Australia, New South Wales[3] | EF534038 |
| *Chromodoris aspersa* | -- | -- | Australia, Queensland[3] | EF534026 |
| *Chromodoris epicuria* | Australia, Tasmania[3] | EF535114 | -- | -- |
| *Chromodoris geometrica* | -- | -- | Australia, Queensland[3] | EF534029 |
| *Chromodoris krohni* | Spain, NE Atlantic[4] | AY345036 | Spain, NE Atlantic[2] | AJ224774 |
| *Chromodoris kuiteri* | Australia, Queensland[5] | AF249804 | Australia, Queensland[5] | AF249214 |

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|---|---|---|---|---|
| *Chromodoris kuniei* | Australia, Queensland[3] | EF535112 | Australia, Queensland[3] | EF534033 |
| *Chromodoris leopardus* | Australia, Queensland[3] | EF535116 | -- | -- |
| *Chromodoris lochi* | -- | -- | Australia, Queensland[3] | EF534027 |
| *Chromodoris luteorosa* | Spain, NE Atlantic[1] | AJ223259 | -- | -- |
| *Chromodoris magnifica* | Australia, Queensland[3] | EF535110 | Australia, Queensland[3] | EF534028 |
| *Chromodoris purpurea* | Spain, NE Atlantic[1] | AJ223260 | -- | -- |
| *Chromodoris quadricolor* | Egypt, Red Sea[5] | AF249802 | Egypt, Red Sea[2] | AJ224773 |
| *Chromodoris splendida* | Australia, Queensland[3] | EF535115 | -- | -- |
| *Chromodoris striatella* | Australia, Queensland[3] | EF535111 | -- | -- |
| *Chromodoris tasmaniensis* | Australia, Tasmania[3] | EF535113 | Australia, Tasmania[3] | EF534032 |
| *Chromodoris tinctoria* | -- | -- | Korea[13] | AF188676 |
| *Cratena peregrina* | Spain, Mediterranean Sea[5] | AF249786 | -- | -- |
| *Cratena pilata* | USA, South Carolina* | GQ292053 | -- | -- |
| *Crimora papillata* | Spain, Mediterranean Sea[5] | AF249821 | -- | -- |
| *Cuthona caerulea* | North Sea[5] | AF249807 | North Sea[5] | AF249199 |
| *Cuthona cocachroma* | USA, Washington* | GQ292071 | USA, Washington* | GQ326893 |
| *Cuthona concinna* | USA, Washington* | GQ292072 | USA, Washington* | GQ326898 |
| *Cuthona nana* | -- | -- | North Sea[7] | AY165760 |
| *Cuthona ocellata* | Portugal[4] | AY345043 | -- | -- |
| *Cuthona sibogae* | -- | -- | Australia, Queensland[7] | AY165761 |
| *Dendrodoris citrina* | NZ, Auckland# | GQ292043 | NZ, Auckland# | GQ326878 |
| *Dendrodoris denisoni* | NZ, Auckland# | GQ292047 | NZ, Auckland# | GQ326872 |

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|---|---|---|---|---|
| *Dendrodoris fumata* | -- | -- | Australia, Queensland[5] | AF249216 |
| *Dendrodoris nigra* | -- | -- | Australia, Queensland[5] | AF249215 |
| *Dendrodoris nigra* | -- | -- | NZ, Auckland[#] | GQ326871 |
| *Dendronotus albopunctatus* | USA, Washington* | GQ292064 | USA, Washington* | GQ326861 |
| *Dendronotus dalli* | USA, North Atlantic[5] | AF249800 | USA, North Atlantic[7] | AY165757 |
| *Dendronotus frondosus* | Sweden[1] | AJ223261 | North Sea[5] | AF249206 |
| *Dendronotus frondosus* | USA, Washington* | GQ292063 | USA, Washington* | GQ326860 |
| *Dendronotus iris* | USA, Washington* | GQ292062 | Canada, Nunavut[7] | AY165758 |
| *Dendronotus* sp. 1 | USA, Washington* | GQ292061 | -- | -- |
| *Dermatobranchus semistriatus* | -- | -- | Australia, Queensland[5] | AF249195 |
| *Diaphorodoris lirulatocauda* | USA, Washington* | GQ292027 | -- | -- |
| *Diaphorodoris luteocincta* | Spain, NE Atlantic[5] | AF249796 | Spain, NE Atlantic[2] | AJ224775 |
| *Diaulula sandiegensis* | USA, Washington* | GQ292030 | USA, Washington* | GQ326865 |
| *Diaulula* sp. | -- | -- | USA, California[9] | AY145374 |
| *Digidentis cf. arbutus* | Australia, Tasmania[3] | EF535143 | Australia, Tasmania[3] | EF534015 |
| *Digidentis perplexa* | Australia, Tasmania[3] | EF535144 | -- | -- |
| *Dirona albolineata* | USA, Washington* | GQ292058 | USA, Washington* | GQ326888 |
| *Dirona picta* | USA, California[13] | DQ026831 | -- | -- |
| *Discodoris atromaculata* | Turkey, Mediterranean Sea[5] | AF249784 | Spain, Mediterranean Sea[10] | AF120521 |
| *Discodoris concinna* | Australia, Queensland[5] | AF249801 | Australia, Queensland[5] | AF249213 |
| *Discodoris concinna* | -- | -- | Dominican Republic[2] | AJ224781 |

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|---|---|---|---|---|
| *Discodoris confusa* | Madeira, Portugal[4] | AY345040 | -- | -- |
| *Diversidoris aurantinodulosa* | Australia, Queensland[3] | EF535141 | Australia, Queensland[3] | EF534011 |
| Dorid Unknown A | Antarctica, Ross Sea* | GQ292046 | Antarctica, Ross Sea* | GQ326877 |
| Dorid YB | Antarctica, Ross Sea* | GQ292044 | Antarctica, Ross Sea* | GQ326879 |
| *Doriopsilla areolata* | Spain, NE Atlantic[1] | AJ223262 | -- | -- |
| *Doriopsis granulosa* | Australia, Queensland[5] | AF249798 | Australia, Queensland[5] | AF249212 |
| *Doris* (was Austrodoris) *kerguelenensis* | Antarctica, Weddell Sea[5] | AF249780 | Antarctica, Weddell Sea[2] | AJ224771 |
| *Doris kerguelenensis* | Antarctica, Ross Sea* | GQ292035-39 | Antarctica, Ross Sea* | GQ326873-75 |
| *Doto antarctica* | Antarctica, Ross Sea* | GQ292025 | Antarctica, Ross Sea* | GQ326882 |
| *Doto columbiana* | USA, Washington* | GQ292026 | USA, Washington* | GQ326881 |
| *Doto coronata* | North Sea[5] | AF249794 | North Sea[5] | AF249203 |
| *Doto eireana* | -- | -- | Spain, NE Atlantic[5] | AF249204 |
| *Doto floridicola* | Spain, Mediterranean Sea[5] | AF249820 | Spain, Mediterranean Sea[7] | AY165759 |
| *Doto koenneckeri* | Spain, NE Atlantic[5] | AF249797 | Spain, NE Atlantic[5] | AF249205 |
| *Doto pinnatifida* | Spain, NE Atlantic[5] | AF249793 | Spain, NE Atlantic[5] | AF249202 |
| *Durvilledoris pusilla* | Papua New Guinea[1] | AJ223269 | -- | -- |
| *Durvilledoris similaris* | Australia, Queensland[3] | EF535128 | -- | -- |
| *Elysia timida* (SAC) | Spain, Mediterranean Sea[5] | AF249818 | -- | -- |
| *Ercolania felina* (SAC) | -- | -- | NZ, Auckland# | GQ326880 |
| *Eubranchus exiguus* | North Sea[5] | AF249792 | North Sea[5] | AJ224787 |
| *Eubranchus rustyus* | USA, Washington* | GQ292065 | USA, Washington* | GQ326905 |

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|---|---|---|---|---|
| *Eubranchus sanjuanensis* | USA, Washington* | GQ292079 | USA, Washington* | GQ326909 |
| *Eubranchus* sp. | Spain, NE Atlantic[5] | AF249791 | Spain, NE Atlantic[5] | AJ224786 |
| *Facelina bostoniensis* | Scotland[4] | AY345031 | Baltic Sea[7] | AY165763 |
| *Facelina punctata* | Spain, Mediterranean Sea[5] | AF249816 | -- | -- |
| *Favorinus brachialis* | Scotland[4] | AY345042 | -- | -- |
| *Flabellina affinis* | Spain, Mediterranean Sea[5] | AF249783 | Spain, Mediterranean Sea[7] | AY165767 |
| *Flabellina amabilis* | USA, Washington* | GQ292022 | USA, Washington* | GQ326912 |
| *Flabellina babai* | -- | -- | Spain, Mediterranean Sea[7] | AY165768 |
| *Flabellina ischitana* | Spain, Mediterranean Sea[5] | AF249814 | -- | -- |
| *Flabellina pedata* | North Sea[5] | AF249817 | North Sea[2] | AJ224788 |
| *Flabellina* sp. | -- | -- | Australia, Queensland[7] | AY165769 |
| *Flabellina trilineata* | USA, Washington* | GQ292024 | USA, Washington* | GQ326911 |
| *Flabellina trophina* | USA, Washington* | GQ292023 | USA, Washington* | GQ326910 |
| *Flabellina verrucosa* | Sweden[11] | AB180830 | USA, North Atlantic[5] | AF249198 |
| *Geitodoris heathi* | USA, Washington* | GQ292031 | USA, Washington* | GQ326862 |
| *Glossodoris atromarginata* | Australia, Queensland[5] | AF249789 | Australia, Queensland[5] | AF249211 |
| *Glossodoris cincta* | Australia, Queensland[3] | EF535136 | Australia, Queensland[3] | EF534034 |
| *Glossodoris edmundsi* | São Tomé, Africa[3] | EF535133 | -- | -- |
| *Glossodoris pallida* | Australia, Queensland[3] | EF535138 | Australia, Queensland[3] | EF534023 |
| *Glossodoris pullata* (was hikuerensis) | Australia, Queensland[3] | EF535137 | Australia, Queensland[3] | EF534024 |

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|---|---|---|---|---|
| *Glossodoris sedna* | USA, Florida Keys[3] | EF535134 | -- | -- |
| *Glossodoris sibogae* | French Polynesia[3] | EF535135 | -- | -- |
| *Godiva banyulensis* | Spain, Mediterranean Sea[5] | AF249782 | Spain, Mediterranean Sea[7] | AY165764 |
| *Goniodoris castanea* | Sweden[1] | AJ223263 | -- | -- |
| *Goniodoris nodosa* | Sweden[1] | AJ223264 | Spain, NE Atlantic[2] | AJ224783 |
| *Halgerda albocristata* | New Caledonia[6] | AY128130 | -- | -- |
| *Halgerda aurantiomaculata* | New Caledonia[6] | AY128132 | -- | -- |
| *Halgerda batangas* | Philippines[6] | AY128135 | -- | -- |
| *Halgerda carlsoni* | New Caledonia[6] | AY128136 | -- | -- |
| *Halgerda cf. willeyi* | Japan, Okinawa[6] | AY128143 | -- | -- |
| *Halgerda diaphana* | Japan, Okinawa[6] | AY128137 | -- | -- |
| *Halgerda gunnessi* | Western Australia[6] | AY128138 | -- | -- |
| *Halgerda malesso* | USA, Guam[6] | AY128139 | -- | -- |
| *Halgerda okinawa* | Japan, Okinawa[6] | AY128140 | -- | -- |
| *Halgerda* sp. | New Caledonia[6] | AY128128 | -- | -- |
| *Halgerda tessellata* | New Caledonia[6] | AY128141 | -- | -- |
| *Halgerda theobroma* | Western Australia[6] | AY128142 | -- | -- |
| *Halgerda willeyi* | Philippines[6] | AY128129 | -- | -- |
| *Hancockia uncinata* | Portugal[4] | AY345047 | -- | -- |
| *Hermissenda crassicornis* | USA, Washington* | GQ292054 | -- | -- |
| *Hypselodoris bennetti* | Australia, New South Wales[3] | EF535131 | Australia, New South Wales[3] | EF534019 |
| *Hypselodoris bilineata* | Madeira, Portugal[3] | EF535125 | -- | -- |
| *Hypselodoris elegans* | Spain, NE Atlantic[5] | AF249787 | Spain, NE Atlantic[2] | AJ224779 |
| *Hypselodoris obscura* | Australia, Queensland[3] | EF535130 | Australia, | EF534012 |

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|---|---|---|---|---|
| | | | Queensland[3] | |
| *Hypselodoris orsinii* | Spain, NE Atlantic[1] | AJ223265 | -- | -- |
| *Hypselodoris villafranca* | -- | -- | Spain, NE Atlantic[2] | AJ224780 |
| *Hypselodoris zephyra* | Australia, Queensland[3] | EF535129 | Australia, Queensland[3] | EF534013 |
| *Janolus cristatus* | North Sea[5] | AF249813 | North Sea[5] | AF249194 |
| *Janolus fuscus* | USA, Washington* | GQ292048 | USA, Washington* | GQ326887 |
| *Jorunna tomentosa* | Sweden[1] | AJ223267 | North Sea[5] | AF249210 |
| Lei | Antarctica, Ross Sea* | GQ292075-77 | Antarctica, Ross Sea* | GQ326906 |
| *Limacia clavigera* | Sweden[1] | AJ223268 | Spain, NE Atlantic[2] | AJ224778 |
| *Limacia jansi* | Costa Rica[12] | DQ231009 | -- | -- |
| *Limapontia nigra* (SAC) | -- | -- | North Sea[2] | AJ224920 |
| *Marionia blainvillea* | Spain, Mediterranean Sea[5] | AF249812 | -- | -- |
| *Melibe leonina* | USA, Washington* | GQ292059 | USA, North Atlantic[2] | AJ224784 |
| *Mexichromis festiva* | Australia, New South Wales[3] | EF535124 | -- | -- |
| *Mexichromis kempfi* | USA, Florida Keys[3] | EF535121 | -- | -- |
| *Mexichromis macropus* | Western Australia[3] | EF535123 | Western Australia[3] | EF534016 |
| *Mexichromis porterae* | USA, California[3] | EF535139 | USA, California[3] | EF534014 |
| Mystery Aeolid | USA, Washington* | GQ292074 | USA, Washington* | GQ326892 |
| *Nembrotha cf. rutilans* | Western Australia[12] | DQ231008 | -- | -- |
| *Nembrotha chamberlaini* | Philippines[12] | DQ230995 | -- | -- |
| *Nembrotha cristata* | Philippines[12] | DQ231003 | -- | -- |
| *Nembrotha kubaryana* | Philippines[12] | DQ231007 | -- | -- |
| *Nembrotha lineolata* | Australia[12] | DQ230995 | -- | -- |
| *Nembrotha mullineri* | Philippines[12] | DQ231004 | -- | -- |

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|---|---|---|---|---|
| Nota Light | Antarctica, Ross Sea* | GQ292069 | Antarctica, Ross Sea* | GQ326901 |
| Nota Small | Antarctica, Ross Sea* | GQ292067 | Antarctica, Ross Sea* | GQ326900 |
| *Notaeolidia depressa* | -- | -- | Antarctica, Weddell Sea[7] | AY165770 |
| *Notaeolidia depressa* | Antarctica, Ross Sea* | GQ292057 | Antarctica, Ross Sea* | GQ326886 |
| *Notodoris citrina* | Western Australia[12] | DQ231010 | -- | -- |
| *Noumea haliclona* | Australia, New South Wales[3] | EF535117 | Australia, New South Wales[3] | EF534037 |
| *Okenia aspersa* | Sweden[1] | AJ223270 | -- | -- |
| *Onchidoris bilamellata* | -- | -- | North Sea[2] | AJ224776 |
| *Onchidoris muricata* | Sweden[1] | AJ223271 | -- | -- |
| *Palio dubia* | Sweden[1] | AJ223272 | -- | -- |
| *Pectenodoris trilineata* | Australia, Queensland[3] | EF535122 | Australia, Queensland[3] | EF534017 |
| *Phestilla lugubris* | USA, Guam[8] | DQ417300 | -- | -- |
| *Phestilla melanobranchia* | Palau[8] | DQ417282 | -- | -- |
| *Phestilla minor* | Palau[8] | DQ417313 | -- | -- |
| *Phestilla* sp. 2 | Palau[8] | DQ417286 | -- | -- |
| *Phidiana lynceus* | -- | -- | Curaçao[7] | AY165765 |
| *Phyllidia coelestis* | -- | -- | Australia, Queensland[5] | AF249209 |
| *Phyllidia elegans* | Papua New Guinea[1] | AJ223276 | -- | -- |
| *Phyllidiella pustulosa* | -- | -- | Australia, Queensland[5] | AF249208 |
| *Platydoris argo* | S Spain[4] | AY345037 | -- | -- |
| *Plocamopherus ceylanicus* | -- | -- | Australia, Queensland[5] | AF249207 |

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|---|---|---|---|---|
| *Polycera aurantiomarginata* | Spain, NE Atlantic[1] | AJ223274 | -- | -- |
| *Polycera quadrilineata* | Sweden[1] | AJ223275 | North Sea[2] | AJ224777 |
| *Polycerella emertoni* | Spain, NE Atlantic[1] | AJ223273 | -- | -- |
| Purple | Antarctica, Ross Sea* | GQ292070 | Antarctica, Ross Sea* | GQ326894-97 |
| *Risbecia tryoni* | Indonesia[3] | EF535132 | Indonesia[3] | EF534018 |
| *Roboastra luteolineata* | Australia[12] | DQ231001 | -- | -- |
| *Roboastra tigris* | USA, California[12] | DQ231002 | -- | -- |
| *Rostanga musculata* | NZ, Auckland# | GQ292032 | NZ, Auckland# | GQ326863 |
| *Rostanga pulchra* | USA, California[4] | AY345044 | -- | -- |
| *Rostanga pulchra* | USA, Washington* | GQ292028-29 | USA, Washington* | GQ326864 |
| *Tambja abdere* | Costa Rica[12] | DQ230995 | -- | -- |
| *Tambja ceutae* | Madeira, Portugal[4] | AY345038 | -- | -- |
| *Tambja eliora* | USA, California[12] | DQ230998 | -- | -- |
| *Tambja morosa* | Philippines[12] | DQ230997 | -- | -- |
| *Tergipes tergipes* | Scotland[4] | AY345032 | North Sea[5] | AF249197 |
| *Tethys fimbria* | Spain, NE Atlantic[4] | AY345035 | -- | -- |
| *Thecacera pennigera* | Spain, NE Atlantic[1] | AJ223277 | -- | -- |
| *Thorunna furtiva* | Indonesia[3] | EF535126 | Indonesia[3] | EF534020 |
| *Thuridilla hopei* (SAC) | Australia, Queensland[5] | AF249810 | -- | -- |
| *Triopha catalinae* | USA, California[13] | DQ026830 | USA, North Atlantic[2] | AJ224782 |
| *Triopha catalinae* | USA, Washington* | GQ292040 | -- | -- |
| *Triopha maculata* | USA, California[4] | AY345045 | -- | -- |
| *Tritonia challengeriana* | Antarctica, Ross Sea* | GQ292052 | Antarctica, Ross Sea* | GQ326904 |
| *Tritonia diomedea* | USA, Washington* | GQ292050 | USA, Washington* | GQ326890 |

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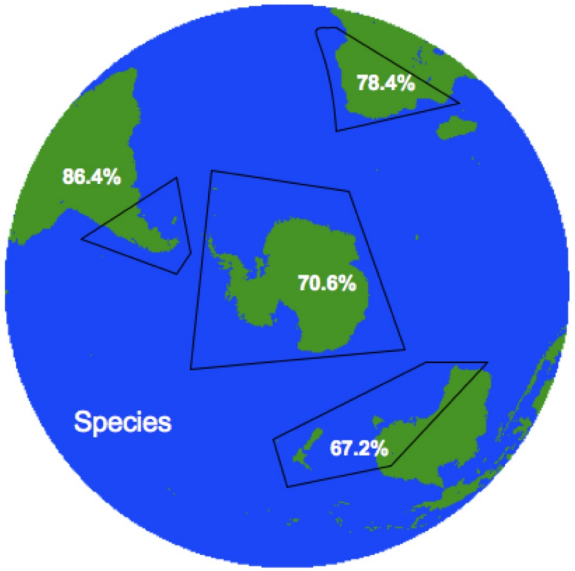
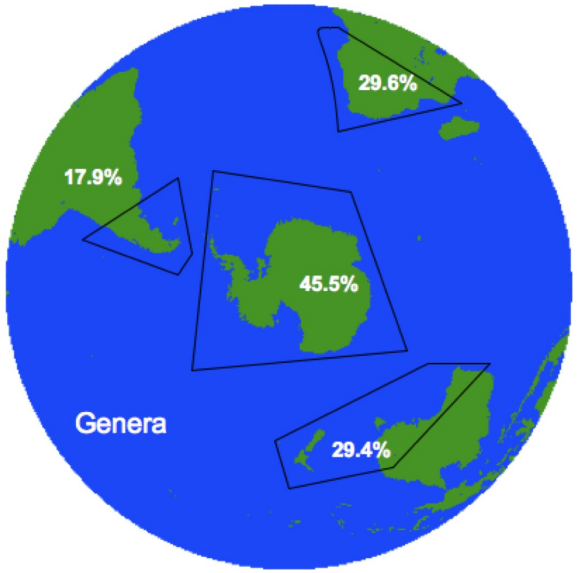
|---|---|---|---|---|
| *Tritonia festiva* | USA, Washington* | GQ292051 | USA, Washington* | GQ326889 |
| *Tritonia nilsodhneri* | -- | -- | Spain, NE Atlantic[5] | AF249200 |
| *Tritonia plebeia* | -- | -- | North Sea[2] | AJ224785 |
| *Tritoniella belli* | -- | -- | Antarctica, Weddell Sea[5] | AF249201 |
| *Tritoniella belli* | Antarctica, Ross Sea* | GQ292056 | Antarctica, Ross Sea* | GQ326883 |
| *Tyrinna nobilis* | Chile[3] | EF535127 | Chile[3] | EF534035 |
| Unknown Aeolid PG | USA, Washington* | GQ292073 | USA, Washington* | GQ326891 |
| Unknown B | Antarctica, Ross Sea* | GQ292068 | Antarctica, Ross Sea* | GQ326899 |
| Unknown D | Antarctica, Ross Sea* | GQ292078 | Antarctica, Ross Sea* | GQ326907-08 |
| Unknown E | Antarctica, Ross Sea* | GQ292066 | Antarctica, Ross Sea* | GQ326902-03 |
| *Verconia verconis* | Australia, New South Wales[3] | EF535118 | Australia, New South Wales[3] | EF534036 |

**Appendix B.** Percentage of endemic taxa in the four outlined southern hemisphere biogeographic regions. A. Shows species level endemism (n= 424). B. Shows genera level endemism (n= 128). Each region outlined by black lines. Published range data on 424 nudibranch species and 128 genera was compiled from each of four Southern Hemisphere continental biogeographic regions: South America, southern Africa, Antarctica, and Australia & New Zealand (Coleman, 1989; Enderby & Enderby, 2005; Gosliner, 1987; Gosliner & Griffiths, 1981; Schrödl, 1999, 2000 & 2003; Wägele, 1987a, 1991 & 1995). Along with the SO, only the temperate waters of the other ocean basins were considered. In the Australia and New Zealand section, only southern Australian species were considered. Those nudibranchs from Queensland or the Great Barrier Reef were ignored as they fit into a different environment with warmer waters and a more tropical climate. Similarly, in southern Africa the sampling was restricted to the cooler waters surrounding South Africa and its neighbors. In South America only the Magellanic (or southern most) province was considered for analogous reasoning. A genus was considered endemic if all of the congeneric species from our analysis were ascribed to the same biogeographic region. Data table included below figure with all Southern Hemisphere occurrences marked with an X. Dataset compiled from available published literature.




a.




b.

| Species | S. Africa | S. America | Antarctica | Austr. & NZ | Other |
|---|---|---|---|---|---|
| Acanthodoris falklandica | | X | | | |
| Acanthodoris globosa | | | | X | |
| Acanthodoris molicella | | | | X | |
| Acanthodoris pilosa | | | | X | |
| Acanthodoris sp. | X | | | | |
| Aegires albus | | | X | | |
| Aegires sp. | X | | | | |
| Aegires villosus | | | | X | X |
| Aeolidia papillosa | | X | | | X |
| Aeolidiella alba | X | | | X | X |
| Aeolidiella faustina | | | | X | |
| Aeolidiella indica | X | | | X | X |
| Aeolidiella multicolor | X | | | | |
| Aeolidiella saldanhensis | X | | | | |
| Aldisa benguelae | X | | | | |
| Aldisa trimaculata | X | | | | |
| Alloiodoris lanuginata | | | | X | |
| Alloiodoris marmorata | | | | X | |
| Amanda armata | X | | | | |
| Ancula fuegiensis | | X | | | |
| Ancula sp. | X | | | | |
| Anisodoris fontani | | X | | | |
| Anisodoris punctuolata | | X | | | |
| Anisodoris sp. 1 | X | | | | |
| Anisodoris sp. 2 | X | | | | |

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|---|---|---|---|---|---|
| Aphelodoris brunnea | X | | | | |
| Aphelodoris lawsae | | | | X | |
| Aphelodoris luctuosa | | | | X | |
| Aphelodoris sp. | X | | | | |
| Aphelodoris varia | | | | X | |
| Archidoris fontaini | | X | | | |
| Archidoris wellingtonensis | | | | X | |
| Armina aoteana | | | | X | |
| Armina cygnaea | | | | X | |
| Armina gilchristi | X | | | | |
| Armina variolosa | | | | X | |
| Artachaea sp. | X | | | | |
| Asteronotus cespitosus | | | | X | |
| Atagema carinata | | | | X | |
| Atagema gibba | X | | | | X |
| Atagema molesta | | | | X | |
| Atagema rugosa | X | | | | X |
| Austraeolis cacotica | | | | X | |
| Austraeolis ornata | | | | X | |
| Austrodoris falklandica | | X | | | |
| Austrodoris kerguelensis | | X | X | | |
| Babakina caprinsulensis | | | | X | |
| Baeolidia palythoae | X | | | | |
| Bathydoris clavigera | | | X | | |
| Bathydoris hodgsoni | | | X | | |
| Berghia chaka | X | | | | |
| Berghia rissodominguezi | | X | | | |
| Bonisa nakaza | X | | | | |

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|---|---|---|---|---|---|
| Bornella adamsi | X | | | X | X |
| Bornella anguilla | X | | | X | X |
| Bornella stellifer | | | | X | |
| Cadlina affinis | | | X | | |
| Cadlina georgiensis | | X | | | |
| Cadlina kerguelensis | | | X | | |
| Cadlina magellanica | | X | | | |
| Cadlina sp. 1 | X | | | | |
| Cadlina sp. 2 | X | | | | |
| Cadlina sp. 3 | X | | | | |
| Cadlina sp. 4 | X | | | | |
| Cadlina sparsa | | X | | | |
| Cadlina willani | | | | X | |
| Cadlinella arnatissima | | | | X | X |
| Caldukia albolineata | | | | X | |
| Caldukia rubiginosa | | | | X | |
| Caloria sp. 1 | X | | | | |
| Caloria sp. 2 | X | | | | |
| Caloria sp. 3 | X | | | | |
| Carambe sp. | X | | | | |
| Casella atromarginata | | | | X | X |
| Catriona alpha | | | | X | |
| Catriona casha | X | | | | |
| Catriona columbiana | X | | | | X |
| Catriona sp. | X | | | | |
| Ceratophyllidia africana | X | | | | |
| Ceratosoma amonea | | | | X | |
| Ceratosoma brevicaudatum | | | | X | |

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|---|---|---|---|---|---|
| Ceratosoma carallinum | | | | X | |
| Ceratosoma cornigerum | | | | X | X |
| Ceratosoma sp. | X | | | | |
| Charcotia granulosa | | | X | | |
| Chromodoris africana | X | | | | X |
| Chromodoris alderi | X | | | | X |
| Chromodoris amoena | | | | X | |
| Chromodoris annulata | X | | | | X |
| Chromodoris aspersa | | | | X | |
| Chromodoris aureomarginata | | | | X | |
| Chromodoris aureopurpurea | | | | X | X |
| Chromodoris colemani | | | | X | |
| Chromodoris collingwoodi | | | | X | |
| Chromodoris daphne | | | | X | |
| Chromodoris decora | | | | X | X |
| Chromodoris elizabethina | | | | X | X |
| Chromodoris epicuria | | | | X | |
| Chromodoris fidelis | | | | X | |
| Chromodoris geometrica | X | | | X | X |
| Chromodoris hamiltoni | X | | | | |
| Chromodoris inopinata | X | | | | X |
| Chromodoris inornata | | | | X | X |
| Chromodoris kuiteri | | | | X | |
| Chromodoris lineolata | | | | X | X |
| Chromodoris lochi | | | | X | X |
| Chromodoris loringi | | | | X | |
| Chromodoris marginata | X | | | X | X |
| Chromodoris mariei | | | | X | X |

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|---|---|---|---|---|---|
| Chromodoris odhneri | | | | X | |
| Chromodoris perplexa | | | | X | |
| Chromodoris sp. 1 | | | | X | |
| Chromodoris sp. 1 | X | | | | |
| Chromodoris sp. 2 | | | | X | |
| Chromodoris sp. 2 | X | | | | |
| Chromodoris sp. 3 | X | | | | |
| Chromodoris sp. 4 | | | | X | |
| Chromodoris sp. 4 | X | | | | |
| Chromodoris sp. 5 | | | | X | |
| Chromodoris sp. 5 | X | | | | |
| Chromodoris sp. 6 | | | | X | |
| Chromodoris sp. 6 | X | | | | |
| Chromodoris sp. 7 | X | | | | |
| Chromodoris sp. 8 | X | | | | |
| Chromodoris splendida | | | | X | |
| Chromodoris striatella | | | | X | |
| Chromodoris tasmaniensis | | | | X | |
| Chromodoris tinctoria | | | | X | X |
| Chromodoris vicina | X | | | | |
| Chromodoris victoriae | | | | X | |
| Chromodoris westraliensis | | | | X | |
| Chromodoris youngbleuthi | | | | X | X |
| Corambe lucea | | X | | | |
| Coryphellina sp. | X | | | | |
| Cratena capensis | X | | | | |
| Cratena lineata | | | | X | X |
| Cratena simba | X | | | | |

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|---|---|---|---|---|---|
| Cratena sp. 1 | X | | | | |
| Cratena sp. 2 | X | | | | |
| Cratena sp. 3 | X | | | | |
| Cratena sp. 4 | X | | | | |
| Crimoa lutea | | | | X | |
| Crimora sp. | X | | | | |
| Cuthona annulata | X | | | | X |
| Cuthona georgiana | | X | X | | |
| Cuthona kanga | X | | | | |
| Cuthona odhneri | | X | | | |
| Cuthona ornata | X | | | | X |
| Cuthona sp. 1 | X | | | | |
| Cuthona sp. 2 | | X | | | |
| Cuthona sp. 2 | X | | | | |
| Cuthona sp. 3 | X | | | | |
| Cuthona sp. 4 | X | | | | |
| Cuthona sp. 5 | X | | | | |
| Cuthona speciosa | X | | | | |
| Cuthona valentini | | X | | | |
| Dendrodoris caesia | X | | | | |
| Dendrodoris citrina | | | | X | |
| Dendrodoris denisoni | X | | | X | X |
| Dendrodoris elongata | | | | X | X |
| Dendrodoris guttata | | | | X | X |
| Dendrodoris nigra | X | | | | X |
| Dendrodoris sp. 1 | X | | | | |
| Dendrodoris sp. 2 | X | | | | |
| Dendrodoris tuberculosa | | | | X | X |

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|---|---|---|---|---|---|
| Dermatobranchus ornatus | | | | X | X |
| Dermatobranchus pulcherrimus | | | | X | |
| Dermatobranchus sp. 1 | X | | | | |
| Dermatobranchus sp. 2 | X | | | | |
| Dermatobranchus sp. 3 | X | | | | |
| Dermatobranchus sp. 4 | X | | | | |
| Diaphorodoris sp. | X | | | | |
| Diaulula hispida | | X | | | |
| Diaulula punctuolata | | X | | | |
| Digidentis perplexa | | | | X | |
| Discodoris concinna | | | | X | |
| Discodoris crawfordi | | | | X | |
| Discodoris fragilis | X | | | | X |
| Discodoris palma | | | | X | |
| Discodoris sp. 1 | X | | | | |
| Discodoris sp. 2 | X | | | | |
| Doriopsilla carneola | | | | X | |
| Doriopsilla miniata | X | | | X | X |
| Doriopsilla sp. 1 | X | | | | |
| Doriopsilla sp. 2 | X | | | | |
| Doriopsis flabellifera | | | | X | |
| Doriopsis granulosa | | | | X | X |
| Doriopsis pecten | X | | | X | X |
| Doris falklandica | | X | | | |
| Doris nanula | | | | X | |
| Doris sp. 1 | X | | | | |
| Doris sp. 2 | X | | | | |
| Doris verrucosa | X | | | | |

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|---|---|---|---|---|---|
| Doris violacea | | | | X | |
| Doto coronata | X | | | | X |
| Doto pinnatifida | X | | | | X |
| Doto rosea | X | | | | X |
| Doto sp. 1 | | | | X | |
| Doto sp. 1 | | | | X | |
| Doto uva | | X | | | |
| Durvilledoris lemniscata | X | | | | X |
| Embletonia gracilis | X | | | X | X |
| Eubranchus agrius | | X | | X | |
| Eubranchus falklandicus | | X | | | |
| Eubranchus fuegiensis | | X | | | |
| Eubranchus sp. 1 | X | | | | |
| Eubranchus sp. 2 | X | | | | |
| Eubranchus sp. 3 | X | | | | |
| Facelina olivcea | X | | | | |
| Facelina sp. | X | | | | |
| Favorinus ghanensis | X | | | | |
| Favorinus japonicus | X | | | | X |
| Favorinus tsuruganus | | | | X | X |
| Fiona pinnata | X | | | X | X |
| Flabeliina albomarginata | | | | X | |
| Flabellina albomarginata | | | | X | |
| Flabellina capensis | X | | | | |
| Flabellina falklandica | | X | X | | |
| Flabellina funeka | X | | | | |
| Flabellina rubrolineata | | | | X | X |
| Flabellina sp. 1 | X | | | | |

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|---|---|---|---|---|---|
| Flabellina sp. 2 | X | | | | |
| Flabellina sp. 3 | X | | | | X |
| Galeojanolus ionnae | | | | X | |
| Gargamella immaculata | | X | | | |
| Gargamella sp. 1 | X | | | | |
| Gargamella sp. 2 | X | | | | |
| Geitodoris capensis | X | | | | |
| Geitodoris patagonica | | X | | | |
| Geitodoris sticta | | | | X | |
| Glaucilla marginata | X | | | X | X |
| Glaucus atlanticus | X | | | | X |
| Glossodoris atromarginata | X | | | X | X |
| Glossodoris rufomarginata | | | | X | |
| Glossodoris sp. 1 | X | | | | |
| Glossodoris sp. 2 | X | | | | |
| Glossodoris sp. 3 | X | | | | |
| Glossodoris sp. 4 | X | | | | |
| Godiva quadricolor | X | | | | |
| Goniodoris castanea | X | | | X | X |
| Goniodoris mercurialis | X | | | | |
| Goniodoris sp. | X | | | | |
| Gymnodoris alba | X | | | | X |
| Gymnodoris arnoldi | | | | X | |
| Gymnodoris inornata | X | | | | X |
| Gymnodoris okinawae | X | | | | X |
| Gymnodoris sp. 1 | X | | | | |
| Gymnodoris sp. 2 | X | | | | |
| Gymnodoris striata | | | | X | X |

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|---|---|---|---|---|---|
| Halgerda formosa | X | | | | X |
| Halgerda graphica | | | | X | |
| Halgerda punctata | X | | | | X |
| Halgerda tessellata | | | | X | |
| Halgerda wasinensis | X | | | | |
| Halgerda wileyi | | | | X | |
| Hallaxa gilva | | | | X | |
| Hallaxa sp. | X | | | | |
| Herviella claror | | | | X | |
| Heterodoris antipodes | | | | X | |
| Heteroplocamus pacificus | | | | X | |
| Hexabranchus sanguineus | X | | | X | X |
| Holoplocamus papposus | | X | | | |
| Hoplodoris nodulosa | | | | X | |
| Hypselodoris obscura | | | | X | |
| Hypselodoris bennetti | | | | X | |
| Hypselodoris capensis | X | | | | |
| Hypselodoris carnea | X | | | | |
| Hypselodoris infucata | X | | | | X |
| Hypselodoris maridadilus | X | | | | X |
| Hypselodoris maritima | | | | X | |
| Hypselodoris sp. 1 | X | | | | |
| Hypselodoris sp. 2 | X | | | | |
| Hypselodoris sp. 3 | X | | | | |
| Janolus capensis | X | | | | |
| Janolus eximus | | | | X | |
| Janolus ignis | | | | X | |
| Janolus longidentalus | X | | | | |

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|---|---|---|---|---|---|
| Janolus mokohinau | | | | X | |
| Janolus novozealandicus | | | | X | |
| Janolus sp. 1 | | | | X | |
| Jason mirabilis | | | | X | |
| Jorunna funebris | | | | X | X |
| Jorunna pantherina | | | | X | |
| Jorunna ramicola | | | | X | |
| Jorunna sp. 1 | | | | X | |
| Jorunna tomentosa | X | | | | X |
| Jorunna zania | X | | | | |
| Kalinga ornata | X | | | X | X |
| Kaloplocamus ramosus | X | | | X | X |
| Kaloplocamus sp. | | | | X | |
| Kaloplocamus yatesi | | | | X | |
| Kentrodoris rubescans | | | | X | X |
| Lecithophorus capensis | X | | | | |
| Lecithophorus sp. | X | | | | |
| Leminda millecra | X | | | | |
| Limacia clavigera | X | | | | X |
| Limacia sp. | | | | X | |
| Madrella ferruginosa | | | | X | |
| Marianina rosea | X | | | X | X |
| Marionia cucullata | | X | | | |
| Marionia sp. 1 | X | | | | |
| Marionia sp. 2 | X | | | | |
| Marioniopsis cyanobranchiata | X | | | | X |
| Marioniopsis sp. 1 | | | | X | |
| Melibe australis | | | | X | |

| | | | | | |
|---|---|---|---|---|---|
| Melibe mirifica | | | | X | |
| Melibe rosea | X | | | | |
| Melibe sp. | X | | | | |
| Miamira magnifica | | | | X | X |
| Miamira sinuata | | | | X | |
| Moridilla brockii | X | | | | X |
| Nembrotha kubaryana | | | | X | |
| Nembrotha livingstonei | X | | | X | |
| Nembrotha purpureolineata | X | | | X | X |
| Nembrotha rutilans | | | | X | |
| Nembrotha sp. 3 | | | | X | |
| Neodoris chrysoderma | | | | X | |
| Neodoris claurina | | X | | | |
| Nossis westralis | | | | X | |
| Notaeolidia depressa | | | X | | |
| Notaeolidia gigas | | | X | | |
| Notaeolidia schmekelae | | | X | | |
| Notobryon wardi | X | | | X | X |
| Notodoris citrina | | | | X | X |
| Notodoris gardineri | | | | X | X |
| Noumea flava | | | | X | |
| Noumea purpurea | X | | | | X |
| Noumea sp. | X | | | | |
| Noumea sulphurea | | | | X | |
| Noumea varians | X | | | | X |
| Okadaia elegans | X | | | | X |
| Okadia cinnabarea | | | | X | |
| Okenia amoenula | X | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Okenia angelensis | | X | | | |
| Okenia pellucida | | | | X | |
| Okenia plana | | | | X | X |
| Okenia sp. | X | | | | |
| Paradoris leuca | | | | X | |
| Phestilla melanobranchia | X | | | X | X |
| Phidiana indica | | | | X | X |
| Phidiana lottini | | X | | | |
| Phidiana milleri | | | | X | |
| Phidiana patagonica | | X | | | |
| Phidiana pegasus | | | | X | |
| Phyllidia sp. 1 | X | | | | |
| Phyllidia sp. 2 | X | | | | |
| Phyllidia varicosa | X | | | | X |
| Phyllodesmium cryptica | | | | X | |
| Phyllodesmium horridus | | | | X | |
| Phyllodesmium hyalinum | X | | | | X |
| Phyllodesmium serratum | X | | | X | X |
| Phyllodesmium sp. 1 | | | | X | |
| Phyllodesmium sp. 2 | | | | X | |
| Phyllodesmium sp. 3 | | | | X | |
| Phyllodesmium sp. 4 | | | | X | |
| Phyllodesmium sp. 5 | | | | X | |
| Platydoris cruenta | X | | | X | X |
| Platydoris scabra | | | | X | |
| Platydoris sp. | X | | | | |
| Platydoris sp. 1 | | | | X | |
| Pleurolidia juliae | | | | X | |

| | | | | | |
|---|---|---|---|---|---|
| Plocamophorus imperialis | | | | X | X |
| Plocamophorus maculatus | X | | | | X |
| Plocamophorus sp. | X | | | | |
| Polycera capensis | X | | | X | |
| Polycera hedgpethi | X | | | X | X |
| Polycera marplatensis | | X | | | |
| Polycera priva | | X | | | |
| Polycera quadrilineata | X | | | | X |
| Polycera risbeci | | | | X | X |
| Polycera sp. | X | | | | |
| Pruvotfolia pselliotes | X | | | | X |
| Pseudotritonia gracilidens | | | X | | |
| Pseudotritonia quadrangularis | | | X | | |
| Pseudovermis hancocki | | | | X | |
| Pteraeolidia ianthina | | | | X | X |
| Risbecia pulchella | X | | | | X |
| Roboastra gracilis | X | | | X | X |
| Roboastra luteolineata | X | | | | X |
| Rostanga arbutus | | | | X | |
| Rostanga muscula | X | | | X | X |
| Rostanga pulchra | | X | | | X |
| Rostanga sp. 1 | X | | | | |
| Rostanga sp. 2 | X | | | | |
| Rostanga sp. 3 | X | | | | |
| Sclerodoris apiculata | X | | | | X |
| Sclerodoris coriacea | X | | | | X |
| Sclerodoris sp. | X | | | | |
| Spurilla alba | | | | X | |

| | | | | | |
|---|---|---|---|---|---|
| Spurilla australis | | | | X | |
| Spurilla macleayi | | | | X | |
| Spurilla neapolitana | | X | | | |
| Tambja affinis | | | | X | X |
| Tambja capensis | X | | | | |
| Tambja morosa | X | | | X | X |
| Tambja sp. | X | | | | |
| Tambja verconis | | | | X | |
| Telarma antarctica | | | X | | |
| Tergipes tergipes | X | | | | X |
| Thecacera darwini | | X | | | |
| Thecacera pacifica | X | | | X | X |
| Thecacera pennigera | X | | | X | X |
| Thecacera sp. | X | | | | |
| Thordisa sp. 1 | X | | | | |
| Thordisa sp. 2 | X | | | | |
| Thorunna horologica | X | | | | |
| Trapania brunnea | | | | X | |
| Trapania rudmani | | | | X | |
| Trapania sp. 1 | X | | | | |
| Trapania sp. 2 | X | | | | |
| Trinchesia reflexa | | | | X | |
| Trinchesia scintillans | | | | X | |
| Trinchesia zelandica | | | | X | |
| Tritonia challengeriana | | X | X | | |
| Tritonia flemingi | | | | X | |
| Tritonia incerta | | | | X | |
| Tritonia nilsodhneri | X | | | | X |

| | | | | | |
|---|---|---|---|---|---|
| Tritonia odhneri | | **X** | | | |
| Tritonia sp. 1 | | **X** | | | |
| Tritonia sp. 1 | | | | **X** | |
| Tritonia sp. 1 | **X** | | | | |
| Tritonia sp. 2 | **X** | | | | |
| Tritonia vorax | | **X** | **X** | | |
| Tyrinna nobilis | | **X** | | | |

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